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14. ABSTRACT The goal of this training grant project is to determine whether the prevalence of ATM carriers among prostate cancer patients treated with radiotherapy that develop erectile dysfunction and urinary morbidity is greater than the prevalence of ATM heterozygosity among patients that do not develop this complication. Regardless of the scientific outcome of the proposal the PI will be left with a vast experience in translational research from which to form new hypotheses and research strategies as he begins his career as an independent physician scientist. To assure a well-rounded experience, the school of medicine will insure that the PI will participate for the first two years of the funded period in Mount Sinai's rigorous clinical research training program. The NIH sponsored program will give the PI formal instruction in Clinical Research and Policy Evaluation, Epidemiology and Biostatistics, Basic Science for the Clinical Investigator, Cultural, Illness, and Community Health Outcomes, Behavioral Medicine, and Ethical Issues in Clinical Research. Also the PI, while at Mount Sinai, will make significant progress in establishing collaborative relationships with well-established prostate cancer researchers and will continue this approach in order to expand the scope of the outlined proposal throughout the funding period of this grant.

15. SUBJECT TERMS
Radiotherapy, Mutation Screening, Functional Analysis

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INTRODUCTION:

A significant proportion of prostate cancer patients treated with radiotherapy develop erectile dysfunction and urinary morbidity induced by exposure to a high dose of radiation. In some cases there are explanations for these reactions, such as doses to large volumes of normal tissue or pre-existing medical conditions such as diabetes or collagen vascular diseases. However, there exists an important subset of patients with no clear explanation for excessive post-treatment morbidity and the potential for a genetic basis must be considered. The purpose of this study was to investigate whether the ATM gene plays a role in this radiation sensitivity. This gene was selected, as the protein it encodes, plays a critical role in the response of cells to irradiation and the repair of radiation-induced damage. Furthermore, cells possessing one mutated copy of this gene are radiosensitive. In addition, the results of a pilot study screening breast cancer patients are supportive of the hypothesis that patients who are carriers of an ATM mutation are more likely to develop radiation-induced complications.

The principal goal of this project was to determine whether men who inherit a mutated copy of the ATM gene are more prone to the development of radiation-induced erectile dysfunction and urinary morbidity. This was accomplished through comprehensive screening of the ATM gene for germline mutations. A correlation was found between radiosensitivity and ATM heterozygosity, and this indicates that possession of a mutated copy of the ATM gene results in susceptibility to complications for prostate cancer radiotherapy patients. In addition, it was found that there was no pathogenic consequence for each identified clinical ATM mutation through the use of functional studies that examined the ability of the ATM protein to act normally in cells from patients who are carriers of a mutation in this gene. This project represents the first completed study to use the powerful DHPLC mutation screening technique to investigate the association between possession of a mutated ATM gene and both erectile dysfunction and the entire clinical course of a patient's urinary morbidity after treatment with radiation for prostate cancer. It is also the first study to examine whether there was a correlation between the presence of a mutation, development of a radiation-induced complication, and impairment of ATM protein function based upon cellular and molecular analyses.

BODY:

My final report covers the period from 2/01/04 to 8/11/08. I successfully completed the Mount Sinai Clinical Research Training Program, which is sponsored by an NIH K30 Clinical Research Curriculum Award, on 5/30/06. In addition to the training plan, regarding the Clinical Research Training Program, I completed additional coursework offered by Mount Sinai Medical School and was conferred a masters degree in Clinical Research on May 30, 2006.

I have published several scientific articles based upon the Mount Sinai experience using low dose rate brachytherapy for the treatment of prostate cancer in addition to other research efforts directed at the treatment of both lung and spine cancers. The articles are referenced in appendix A and D as reportable outcomes. In addition to the publication of articles I have with my mentor received funding as a co-investigator for a study entitled," Genome-Wide Association Study to Identify SNPs and CNPs Associated with Development of Radiation Injury in Prostate Cancer Patients Treated with Radiotherapy", by the Department of the Army through the Prostate Cancer Research Program synergy award mechanism. I have opened a new protocol for the retrospective study of lung brachytherapy and para-spinal brachytherapy. In addition, I will be submitting in early spring 2008 a grant application which proposes to prospectively study in a phase II clinical design the predictive value of testing for mutations in the ATM gene prior to the initiation of therapy.

In addition to articles, protocols and grants, I have made several presentations over the last four and half years as an invited speaker and in order to present submitted research papers which I expect to publish in the coming year, they are enumerated in appendix B as those accepted for formal oral presentations and in appendix C as abstracts presented as either posters, poster discussion and oral presentations. In appendix D are listed research accomplishments directly related to the activities outlined in my training grant.

Based upon the abstracts submitted this year and the *works in progress* at this point, I expect to be able to complete the aims of my research proposal and have them largely published as outlined in the training and to report the results of further granting efforts which should sustain my research efforts into the future.

In addition to positive published research results I have also carried out the functional assays as described in the grant. They are reported in appendix D following the reporting of relevant publications and abstracts related directly to the fulfillment of the grant summarized in tables 1 and 2.

KEY RESEARCH ACCOMPLISHMENTS:

Completed 24 months of coursework required for Clinicial Research Training Program and received a Masters degree from the Mount Sinai School of Medicine.

I have published in 2007 year 8 collaborative works with my clinical mentors Richard Stock, M.D., and Barry Rosenstein,PhD., regarding the natural history of prostate cancer treated with brachytherapy and the genetics of radiosensitivity.

I have established a collaborative effort with the Mount Sinai Department of Urology, which has resulted in the formulation of a research question for which we are actively seeking funding through the NIH and DOD.

I have given 6 oral presentations at major research meetings throughout the radiation and urological community in America, Japan and Germany.

I presented my findings regarding my research efforts at an oral presentation at the American Society of Therapeutic Radiation Oncology (ASTRO) annual meeting in Los Angeles, California in November of 2007, and at least partly as a result of my efforts 9 other residents, fellows and faculty presented work which I either inspired or collaborated extensively in regarding its content and formulation.

Our research team has been awarded to pursue a project entitled," Genome-Wide Association Study to Identify SNPs and CNPs Associated with Development of Radiation Injury in Prostate Cancer Patients Treated with Radiotherapy" through the Department of the Army Prostate Cancer Research Program Synergism Award Mechanism.

Presentation of my research findings in Poster format at the Innovative Minds in Prostate Cancer Today (IMPaCT) meeting September 5-7, 2007

RESULTS OF FUNCTIONAL ASSAYS

An additional goal of this study was to perform functional assays to determine the effect of *ATM* sequence variants on the function of the ATM protein. This research was accomplished using lymphoblastoid cell lines derived from EBV transformed lymphocytes obtained from five subjects who did not exhibit late responses and did not possess *ATM* genetic alterations. For experiments in which p53 phosphorylation was measured, cells were irradiated with either 0 or 4 Gy of x-rays and incubated either 0.5 or 2 hr. The densitometric results for each time point were divided by the value in each experiment for unirradiated cells to normalize these results. Each irradiation was performed a total of three times. The mean values (with standard deviations) for wild type cells incubated either 0.5 or 2.0 hr were 3.2+1.7 or 6.9+3.1, respectively. The results for the cell lines possessing variants are shown in Tables 1 and 2. In addition, ATM

protein levels were measured in each cell line in three separate experiments and divided by the average value obtained for the five wild type *ATM* cell lines.

Table 1. Functional Assays of Lymphoblastoid Cells Derived from Subjects Possessing *ATM* Variants

Cell Line	Radio-sensi-tive Yes/No	Nucleotide Change	Amino Acid Substitu-tion	ATM level	Phospho-p53 0.5 hr	Phospho-p53 2 hr	Normalized α-value
MS01-33	no	4138 C>T	1380 H>Y	1.1+0.6	5.1+4.4	5.4+3.0	1.2±0.2
MS01-30	No	IVS5-7 C>T 378 T>A 4578 C>T	N/A 126 D>E 1526 P>P	0.5+0.3	2.0+1.7	4.5+4.0	1.0±0.4
MS01-39	Yes	5557 G>A 5558 A>T	1853 D>N 1853 D>V	1.3±0.9	1.4±1.0	2.7±0.4	1.1±0.5
MS01-45	No	5557 G>A	1853 D>N	0.4±.04	1.4±.0.6	1.6±1.0	1.3±0.1
MS01-51	Yes	IVS5-7C>T 378 T>A	N/A 126 D>E	0.7±0.5	2.5±2.6	9.5±4.5	0.5±.2
MS01-37	Yes	378 T>A 1176 C>G 4138 C>T	126 D>E 392 G>G 1380 H>Y	1.6±0.2	2.1±1.2	2.0±1.2	1.4±0.4
MS01-67	Yes	4578 C>T	1526 P>P	0.5±.09	4.6±0.8	10.7±3.7	1.2±0.1
MS01-65	No	5557 G>A	1853 D>N	1.1±0.5	2.7±1.2	10.1+4.0	1.2±0.3
Ms01-53	No	378 T>A 1176 C>G	126 D>E 392 G>G	1.0±.0.1	2.5±0.8	6.5±2.1	0.8±0.3
MS01-07	No	4917 G>A 5557 G>A 5558 A>T	1639 P>P 1853 D>N 1853 D>V	0.8±0.5	0.9±0.7	2.0±1.7	0.5±0.3
MS01-37	Yes	378 T>A 1176 C>G 4138 C>T	126 D>E 392 G>G 1380 H>Y	1.6+0.2	2.1+1.2	2.0+1.2	1.4±0.2
MS02-13	YES	378 T>A 6176 C>T	126 T>A 2059 T>I	1.0+0.2	2.0+1.3	5.1+3.7	1.2±0.2
MS02-73	YES	IVS62+8 A>C	N/A	0.8+0.3	4.5+4.0	3.8+1.3	0.8±0.3
MS01-87	YES	5071 A>C	1691 S>R	1.0+0.5	2.3+1.3	5.0+2.0	0.8±0.1
MS01-03	NO	2614 C>T 2685 A>C	872 P>S 895 L>L	0.7+0.2	1.1+0.8	1.5+0.4	1.2±0.6
MS01-35	NO	1229 T>C	410 V>A	0.9+0.6	2.9+0.1	5.7+3.7	1.1±0.2
MS02-34	YES	915 G>C	25 R>P	2.0+1.5	1.5+0.6	1.8+1.1	1.3±0.5
MS02-05	YES	NONE	N/A	0.7+0.4	3.1+3.7	4.6+3.7	1.1±0.3
MS03-13	YES	NONE	N/A	0.7+0.1	3.6+1.2	7.9+3.8	0.9±0.5
MS03-48	YES	NONE	N/A	0.5+0.3	2.4+1.4	6.8+2.1	1.3±0.1

Table 2. Functional Assays of *ATM* Homozygoyte and *ATM* Heterozygote Lymphoblastoid Cell Lines

Cell Line	Homozygote or Heterozygote	ATM Level	Phospho p53 0.5 hr	Phospho p53 2 hr	Normalized α-value for radiation survival curve
8388	heterozygote	0.7±0.6	1.6±0.2	6.7±2.3	1.5±0.2
8925	heterozygote	0.7±0.8	1.9±0.4	5.1±0.1	1.4±0.2
8928	heterozygote	0.8±0.3	3.8±3.5	3.5±2.7	1.7±0.2
9579	heterozygote	0.5±0.3	2.3±1.3	2.6±0.3	1.1±0.3
2781	heterozygote	0.7±0.5	3.2±0.6	4.5±4.1	1.6±0.2
9588	heterozygote	0.5±0.5	6.1±4.0	6.9±2.8	1.2±0.3
8436	homozygote	0.04±0.06	2.9±1.2	2.8±0.4	1.8±0.3
9581	homozygote	0.08±0.02	1.5±1.7	4.0±1.6	2.0±0.3
9582	homozygote	0.05±0.02	2.0±4.4	2.1±0.4	2.2±0.3
2782	homozygote	0.08±0.05	2.1±3.1	3.1±1.3	2.1±0.3
1525	homozygote	0.05±0.02	2.6±1.1	3.1±1.2	1.8±0.2
11254	homozygote	0.09±0.06	1.8±0.1	2.5±0.9	2.3±0.3
9586	homozygote	0.24±0.22	1.7±1.0	4.3±1.9	1.8±0.4
13328	homozygote	0.13±0.09	0.6±0.5	2.1±1.3	2.1±0.3

The results for cells derived from AT patients clearly show a significantly lower level of ATM protein in these cells compared with wild type cells. In addition, the levels of p53 phosphorylation are consistently lower than those detected in wild type cells. The ATM levels are also consistently lower in the heterozygotes and the levels of phosphorylated p53 are also generally lower, although none of these values differed significantly from those obtained for wild type cells due to the variation in the results between experiments. There was a variation among the cell lines, but no clear pattern emerged that correlated either with the possession of an *ATM* variant (including the 5557 SNP) or whether the patient developed a late radiotherapy reaction. Hence, the results of this work suggest that neither measurement of ATM levels nor p53 phosphorylation can serve as a predictor as to whether the patient will develop late morbidity following radiotherapy.

The radiosensitivity of each cell line was also determined from the growth response of cells irradiated with either 0, 0.5, 1.0 or 2.0 Gy of X-rays by extrapolating the growth curve to the intercept at zero time. The radiosensitivity of each cell line was estimated from the α-value ($S = e^{-\alpha D}$) normalized to the value obtained for wild type cells listed in Tables 1 and 2. The α-values for the cell lines derived from AT patients were all significantly greater than one. In addition, the α-values for the AT heterozygotes were consistently greater than one, although generally not significantly greater. In contrast, the α-values for the cell lines obtained from the breast cancer patients were variable and none was significantly greater than one.

This is not altogether surprising, since clearly none of the patients screened in this study manifested a radiation sensitivity approaching that displayed by a person suffering from AT. Any radiosensitive patients likely have only a mild radiosensitivity. However, even a slight radiosensitivity is probably sufficient to result the development of a late response since the dose used in treating breast cancer represents the tolerance dose. Hence, even just a 5-10% increase in radiosensitivity will make the difference as to whether a person will or will not develop a radiation complication. It is likely that the subtle changes in ATM protein function that result from the variants identified in this study are sufficient to cause these types of very mild changes in protein function. In contrast, it is impossible with the techniques currently available to detect such small changes in ATM function using the westerns performed in this work to measure ATM levels and p53 phosphorylation. Hence, the results of this study indicate that the identification of genetic variants will serve as a far more important basis of a predictive assay for radiosensitivity compared with functional assays.

Key Research Accomplishments

- No significant differences were detected in any of the functional end-points measured between patients who developed late complications compared with those that did not exhibit this type of radiation-induced morbidity. In addition, no significant differences in the results for the functional assays were identified for any *ATM* variant compared with wild type cells.

REPORTABLE OUTCOMES

Publications 2/2004 to 8/2008:

Stock RG, Cahlon O, Cesaretti JA, Kollmeier MA, Stone NN. "Combined Modality Treatment in the Management of High Risk Prostate Cancer." *Int J Radiat Oncol Biol Phys* 2004 Aug 1; 59(5):1352-1359.

Cesaretti JA, Stone NN, Stock RG. "Does a prior transurethral resection of the prostate compromise brachytherapy quality: a dosimetric analysis." *Int J Radiat Oncol Biol Phys.* 2004 Oct 1; 60(2):648-653.

Cesaretti JA, Stock RG, Atencio DA, Bernstein J, Stone NN, Wallenstein S, Green S, Loeb KL, Kollmeier MA, Smith M, Rosenstein BS. "ATM sequence variants are predictive of adverse radiotherapy response among patients treated for prostate cancer." *Int J Radiat Oncol Biol Phys*. 2005 Jan 1;61(1):196-202.

Cesaretti JA, Stock RG, Stone NN. "Brachytherapy." Book Chapter, Prostate Cancer: Principles and Practice, Ed by Kirby R, Partin AW, Feneley M, Parsons JK. Taylor and Francis Medical Books.

Kollmeier MA, Stock RG, Cesaretti JA, Stone NN. "Urinary Morbidity Following Post-brachytherapy Transurethral Resection of the Prostate." *J Urol*. 2005 Mar;173(3):808-12.

Andreassen CN, Overgaard J, Alsner J, Overgaard M, Herskind C, Cesaretti JA et al. "ATM sequence variants and risk of radiation-induced subcutaneous fibrosis after postmastectomy radiotherapy." *Int J Radiat Oncol Biol Phys*. 2006 Mar 1;64(3):776-83.

Stock RG, Cesaretti JA, Stone NN. "Disease-specific survival following the brachytherapy management of prostate cancer." *Int J Radiat Oncol Biol Phys*. 2006 Mar 1;64(3):810-6.

Andreassen CN, Overgaard J, Alsner J, Overgaard M, Herskind C, Cesaretti JA, Atencio DP, Green S, Formenti SC, Stock RG, Rosenstein BS. "ATM sequence variants and risk of radiation-induced subcutaneous fibrosis after postmastectomy radiotherapy." *Int J Radiat Oncol Biol Phys*. 2006 Mar 1;64(3):776-83.

Stock RG, Stone NN, Cesaretti JA, Rosenstein BS. "Biologically effective dose values for prostate brachytherapy: Effects on PSA failure and post-treatment biopsy results." *Int J Radiat Oncol Biol Phys*. 2006 Feb 1;64(2):527-33.

Stock RG, Ho A, Cesaretti JA, Stone NN. "Changing the patterns of failure for high-risk prostate cancer patients by optimizing local control." *Int J Radiat Oncol Biol Phys*. 2006 Oct 1;66(2):389-94.

Ho AY, Atencio DP, Peters S, Stock RG, Formenti SC, Cesaretti JA, Green S, Haffty B, Drumea K, Leitzin L, Kuten A, Azria D, Ozsahin M, Overgaard J, Andreassen CN, Trop CS, Park J, Rosenstein BS. "Genetic predictors of adverse radiotherapy effects: the Gene-PARE project." *Int J Radiat Oncol Biol Phys*. 2006 Jul 1;65(3):646-55.

Peters CA, Cesaretti JA, Stone NN, Stock RG. "Brachytherapy for patients with inflammatory bowel disease." *Int J Radiat Oncol Biol Phys*. 2006 Oct 1;66(2):424-9.

Lehrer S, Cesaretti JA, Stone NN, Stock RG. "Urinary symptom flare after brachytherapy for prostate cancer is associated with erectile dysfunction and more urinary symptoms before implantation." *BJU Int*. 2006 Nov;98(5):979-81.

Schiff JD, Bar-Chama N, Cesaretti JA, Stock R. "Early use of a phosphodiesterase inhibitor after brachytherapy restores and preserves erectile function." *BJU Int.* 2006 Dec;98(6):1255-8.

Zagar TM, Stock RG, Cesaretti JA, Stone NN. "Assessment of postbrachytherapy sexual function: a comparison of the IIEF-5 and the MSEFS." *Brachytherapy*. 2007 Jan-Mar;6(1):26-33.

Ho AY, Burri RJ, Jennings GT, Stone NN, Cesaretti JA, Stock RG. "Is seminal vesicle implantation with permanent sources possible? A dose-volume histogram analysis in patients undergoing combined 103Pd implantation and external beam radiation for T3c prostate cancer." *Brachytherapy*. 2007 Jan-Mar;6(1):38-43.

Cesaretti JA, Stock RG, Atencio DP, Peters SA, Peters CA, Burri RJ, Stone NN, Rosenstein BS. "A genetically determined dose-volume histogram predicts for rectal bleeding among patients treated with prostate brachytherapy." *Int J Radiat Oncol Biol Phys*. 2007 May 8; [Epub ahead of print]

Ho AY, Fan G, Atencio DP, Green S, Formenti SC, Haffty BG, Iyengar P, Bernstein JL, Stock RG, Cesaretti JA, Rosenstein BS. "Possession of ATM Sequence Variants as Predictor for Late Normal Tissue Responses in Breast Cancer Patients Treated with Radiotherapy." *Int J Radiat Oncol Biol Phys.* 2007 May 19; [Epub ahead of print]

Cesaretti JA, Kao J, Stone NN, Stock RG. "Effect of low dose-rate prostate brachytherapy on the sexual health of men with optimal sexual function before treatment: analysis at >/= 7 years of follow-up." BJU Int. 2007 Aug;100(2):362-7.

Peters CA, Stock RG, Cesaretti JA, Atencio DP, Peters S, Burri RJ, Stone NN, Ostrer H, Rosenstein BS. "TGFB1 Single Nucleotide Polymorphisms are Associated with Adverse Quality of Life in Prostate Cancer Patients Treated with Radiotherapy." Int J Radiat Oncol Biol Phys. 2007 Aug 7; [Epub ahead of print]

Cesaretti JA, Stone NN, Skouteris BM, Park JL, Stock RG. "Brachytherapy for Prostate Cancer." Cancer J. 2007 Sep-Oct;13(5):302-12.

Kao J, Stone NN, Lavaf A, Dumane V, Cesaretti JA, Stock RG. “(125)I monotherapy using D90 implant doses of 180 Gy or greater.” Int J Radiat Oncol Biol Phys. 2007 Oct

Lavaf A, Genden EM, Cesaretti JA, Packer S, Kao J. “Adjuvant radiotherapy improves overall survival for patients with lymph node-positive head and neck squamous cell carcinoma” Cancer. 2007 Dec 12; [Epub ahead of print]

Cesaretti J, Pennather A, Rosenstein B, Swanson S, Fernando H. “Radiosurgery for Thoracic Malignancies.” Ann Thorac Surg. 2008 Jan; [In press]

Presentations in 2/2004 to 8/2008

Cesaretti JA. "Real Time Brachytherapy: The American Experience." International Course on Brachytherapy, San Paolo Hospital, Febuary 2004, Savona, Italy.

Cesaretti JA. "Genetic Associations Are Predictive Of Adverse Outcomes Following Radiotherapy For Prostate Cancer." Radiological and Medical Physics Society of New York (RAMPS), Spring Symposium Advancing Radiation Oncology Planning Through an Understanding of Biology, May 2004, New York, New York.

Cesaretti JA. "ATM Sequence Variants are Predictive of Adverse Radiotherapy Response Among Patients Treated for Prostate Cancer." ASTRO 46th Annual Meeting, October 2004, Atlanta, Georgia.

Cesaretti JA. "Intensity Modulated Radiation Therapy for Brain Malignancies." IV Advanced Techniques and Technology in Image-Guided Brain and Spine Surgery, December 5, 2004, New York, New York.

Cesaretti JA. "Radiation Therapy for Esophageal Carcinoma." From Gastroesophageal Reflux Disease to Esophageal Cancer: New Treatments and Technologies, April 2, 2005, The New York Academy of Medicine, New York, New York.

Cesaretti JA. "Intensity Modulated Radiation Therapy for Prostate Cancer" and "Combined Modality Therapy for Prostate Cancer." Advanced Workshop in the Treatment of Prostate Cancer, April 27-29, 2005, The New York Academy of Medicine, New York, New York.

Cesaretti JA. "Intensity Modulated Radiation Therapy for Prostate Cancer" and "Combined Modality Therapy for Prostate Cancer." Advanced Workshop in the Treatment of Prostate Cancer II, September 27-29, 2005, The New York Academy of Medicine, New York, New York.

Cesaretti JA. "Erectile function following prostate brachytherapy with 7 years minimum follow-up." ASTRO 47th Annual meeting, October 2005, Denver, Colorado.

Cesaretti JA. "The Genetics of Radiation Sensitivity." 11th Annual Scottsdale Prostate Cancer Symposium, March 1-5, 2006, Scottsdale, Arizona.

Cesaretti JA. "A dose volume histogram for the incidence of rectal bleeding among ATM heterozygotes." ASTRO 48th Annual meeting, November 2006, Philadelphia, Pennsylvania.

Cesaretti JA. "Loose versus Stranded seeds." "Genetic Predictors of Radiotherapy Response." "Salvage Brachytherapy." 12th Annual Scottsdale Prostate Cancer Symposium, March 1-4, 2007, Scottsdale, Arizona.

Cesaretti JA. "Prostate Brachytherapy, the Mount Sinai Experience." 95th Annual Japanese Urological Association meeting, April 14th 2007, Osaka, Japan.

Cesaretti JA. "Intensity Modulated Radiation Therapy for Prostate Cancer" and "Combined Modality Therapy for Prostate Cancer." Advanced Workshop in the Treatment of Prostate Cancer II, April 25-27, 2007, The New York Academy of Medicine, New York, New York.

Cesaretti JA. "Stereotactic Radiosurgery for Lung Cancer." 1st Annual Minimally Invasive Thoracic Surgery Summit, June 6th 2007, New York, New York.

Cesaretti JA. "Single Nucleotide Polymorphisms as Predictors for Development of Erectile Dysfunction in African-American Men Treated With Radiotherapy for Prostate Cancer." ASTRO 49th Annual meeting, November 2007, Los Angeles, California.

Cesaretti JA. "Brachytherapy for Prostate Cancer." New York Roentgen Society, November 2007, New York, New York.

Cesaretti JA. "Innovations in Prostate Brachytherapy." 4th International Interstitial Prostate Brachytherapy Teaching Course, January 2008, Bergisch-Gladbach, Germany.

Abstracts accepted for Presentations from 2/2004 to 8/2008

Cesaretti JA, Stock RG, Stone NN and Rosenstein BS "Combined Low Dose Rate Brachytherapy and External Beam Radiotherapy Result in a Favorable Acute Urinary Symptom Profile Relative to Brachytherapy Monotherapy at the Same Biological Equivalent Dose (BED)" International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Page S630

Peters CA, Stone NN, Cesaretti JA and Stock RG "The Effect of Family History on Outcome in Patients Treated With Low-Dose Rate Brachytherapy for Clinically Localized Prostate Cancer" International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Pages S370-S371

Solan AN, Stock RG, Cesaretti JA and Stone NN "Correlation Between Erectile Dysfunction and Dose to Penile Bulb and Neurovascular Bundles Following Prostate Brachytherapy" International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Pages S351-S352

Skouteris VM, Stone NN, Stock RG and Cesaretti JA "Dose Response Study of Pd-103 Prostate Seed Implantation" International Journal of Radiation Oncology*Biology* Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Pages S349-S350

Stock RG, Cesaretti JA and Stone NN "Comparisons of PSA Failure Definitions Following Trimodality Therapy for Intermediate to High-Risk Prostate Cancer" International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Pages S344-S345

Klein TJ, Stock RG, Cesaretti JA and Stone NN "Prognostic Significance of the 5-Year PSA Value for Predicting Prostate Cancer Recurrence Following Brachytherapy" International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Pages S176-S177

Ho AY, Fan G, Cesaretti JA, Stone NN and Stock RG "Young Men have Equivalent Biochemical Outcomes Compared to Older Men After Treatment With Prostate Brachytherapy" International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Pages S90-S91

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Research efforts directly related to the training grant from 2/2004 to 8/2008.

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CONCLUSIONS:

My professional training as a translational scientist with and emphasis on prostate cancer treatment is progressing on several important fronts. I have published several articles in medical research journals this year about prostate cancer treatment outcomes, the side effect profile of prostate cancer treatment and the genetics of radiation sensitivity.

I have completed the K30 Physician Research Training Program and have been conferred a Masters degree in May 2006 in Clinical Research from the Mount Sinai School of Medicine.

The results of my research project were presented at the ASTRO annual meeting in addition to the work of my residents, my research mentors and my junior faculty colleagues.

The research group has been awarded additional funds to study genetic associations between prostate radiotherapy and genetic polymorphisms/ mutations.

REFERENCES: None

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CLINICAL INVESTIGATION **Prostate**

^{125}I MONOTHERAPY USING D90 IMPLANT DOSES OF 180 GY OR GREATER

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Purpose: The purpose of this study was to characterize the oncologic results and toxicity profile of patients treated with ^{125}I implants using the dose delivered to 90% of the gland from the dose–volume histogram (D90) of greater than 144 Gy.

Methods and Materials: From June 1995 to Feb 2005, a total of 643 patients were treated with ^{125}I monotherapy for T1–T2 prostate cancer with a D90 of 180 Gy or greater (median, 197 Gy; range, 180–267 Gy). Implantations were performed using a real-time ultrasound-guided seed-placement method and intraoperative dosimetry to optimize target coverage and homogeneity by using modified peripheral loading. We analyzed biochemical disease-free survival (bDFS) of 435 patients who had a minimum 2-year prostate-specific antigen follow-up (median follow-up, 6.7 years; range, 2.0–11.1 years).

Results: Five-year bDFS rates for the entire cohort using the American Society for Therapeutic Radiology and Oncology and Phoenix definitions were 96.9% and 96.5%, respectively. Using the Phoenix definition, 5-year bDFS rates were 97.3% for low-risk patients and 92.8% for intermediate/high-risk patients. The positive biopsy rate was 4.1%. The freedom rate from Grade 2 or higher rectal bleeding at 5 years was 88.5%. Acute urinary retention occurred in 10.7%, more commonly in patients with high pretreatment International Prostate Symptom Scores ($p < 0.01$). In patients who were potent before treatment, 73.4% remained potent at 5 years after implantation.

Conclusions: Patients with a minimum D90 of 180 Gy had outstanding local control based on prostate-specific antigen control and biopsy data. Toxicity profiles, particularly for long-term urinary and sexual function, were excellent and showed that D90 doses of 180 Gy or greater performed using the technique described were feasible and tolerable. © 2008 Elsevier Inc.

Prostate cancer, Brachytherapy, Dose escalation, Toxicity profile.

INTRODUCTION

Based on correlative studies of prostate-specific antigen (PSA) outcome and postimplantation dosimetry, a dose delivered to 90% of the gland from the dose–volume histogram (D90) of 144 Gy or greater was adopted as the standard of care for ^{125}I monotherapy (1–3). Subsequently, multiple randomized trials and comparative series showed a clear dose response for external beam radiation greater than 70 Gy, even for low-risk subgroups (4–7). There is significant interest in additional dose escalation using three-dimensional external beam radiation or intensity-modulated radiation therapy for patients with prostate cancer (8, 9). Technical advances in the application of image-guided intensity-modulated radiation therapy for the treatment of patients with prostate cancer have increased normal-tissue sparing and decreased the clinical impact of intra- and interfraction organ motion (10, 11). Extremely conformal dose distributions, use of real-time image guidance, and elimination of organ motion by directly placing radioactive sources in the prostate gland are well-known advantages of prostate brachytherapy (12).

Although prostate brachytherapy is a logical approach to further dose escalation to the prostate, the safety and efficacy of dose escalation greater than D90 of 144 Gy using ^{125}I monotherapy has not been clearly established (13, 14). At Mount Sinai School of Medicine, the prescription dose has been a D90 of 160 Gy both before and after implementation of Task Group 43 guidelines using real-time intraoperative planning. This represents an approximate 9% increase in dose over the more commonly used prescription dose of 144 Gy. With the real-time approach, concordance between intraoperative and postimplantation dosimetry is high (15). One advantage of using a real-time approach with a prescription dose of 160 Gy, in contrast to other approaches, is that low-dose implants are extremely rare and high-dose implants are relatively common (16, 17). High-quality implants typically will result in a postimplantation D90 of 112–125% of the prescription dose. For this reason, we undertook this

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study to define the quality of life and PSA outcomes of patients with a D90 of 180 Gy or greater at the time of postimplantation dosimetry.

METHODS AND MATERIALS

From June 1995 to February 2005, a total of 643 patients were treated with ^{125}I monotherapy for T1–T2 prostate cancer with a D90 of 180 Gy or greater (Task Group 43 guidelines; median, 197 Gy; range, 180–267 Gy) and had available clinical follow-up data (Table 1). Pretreatment, no patient had radiologic or pathologic evidence of metastatic disease. Clinical staging was determined using the 2002 American Joint Committee on Cancer recommendations. A seminal vesicle biopsy was performed at the discretion of the referring urologist for patients at increased risk of occult seminal vesicle involvement. In general, this was offered to patients with a Gleason score higher than 6, PSA of 10 ng/ml or greater, or Stage T2b or greater. Median pretreatment PSA was 6.1 ng/ml (range, 0.3–35 ng/ml), median Gleason score was 6 (range, 2–7), and 70% were clinically staged T1a–T1c, whereas 30% were T2a–T2c. Based on the presence of one or more high-risk features (PSA > 10 ng/ml; Gleason score, 7–10; or Stage T2b–T2c disease), patients were classified into prognostic risk groups according to the National Comprehensive Cancer Network (NCCN; www.nccn.org). The majority of patients were considered low risk (85.2%) based on clinical stage of T1–T2a, Gleason score of 2–6, and pretreatment PSA less than 10 ng/ml. The remainder were classified as NCCN intermediate risk (16.6%) on the basis of clinical Stage T2b–T2c, Gleason score of 7, or PSA of 10.1–20 ng/ml or high risk (0.3%) based on PSA greater than 20 ng/ml. There were no patients with Stage T3 or Gleason score 8 to 10 disease treated with ^{125}I monotherapy.

Table 1. Patient characteristics

	No. of patients	Percentage
Clinical stage		
T1a	1	0.2
T1b	2	0.3
T1c	444	69.1
T2a	144	22.4
T2b	46	7.2
T2c	6	0.9
Gleason score		
2	3	0.5
3	2	0.3
4	15	2.3
5	41	6.4
6	581	90.4
7	1	0.2
Pretreatment prostate-specific antigen (ng/ml)	Median 6.1	
0–4	66	10.3
4.1–10	526	81.8
10.1–20	50	7.8
>20	2	0.3
Hormonal therapy		
No	444	69.0
Yes	199	31.0
Previous transurethral resection of the prostate		
No	624	97.0
Yes	19	3.0
Ultrasound volume (ml)	Median 44.0	
14.3–40	268	41.7
40.1–60	283	44.0
60–80	76	11.8
>80	16	2.5
Unknown	1	0.2

Treatment

Prostate seed implantations were performed under spinal anesthesia in the dorsolithotomy position. A transrectal ultrasound and template were used to image relevant anatomy and guide needle placement. The prostate, urethra, bladder, and rectum were contoured on axial slices obtained at 5-mm slices for intraoperative dosimetry. Implantations were performed by using a modified peripheral-loading strategy with real-time ultrasound-guided seed placement and intraoperative dosimetry to optimize target coverage and homogeneity. This technique was developed because of concern about performing preplanned implantation in an organ subject to displacement and distortion by needle placement (18). The real-time method relies on intraoperative planning with axial imaging to place the needles and sagittal imaging to guide seed placement.

Briefly, needles were placed on the largest prostate slice on transverse imaging with 1-cm spacing, with urethral sparing. Total activity implanted per prostate volume was derived from a reference table developed by Anderson (19). Total activity of seeds ordered for each case was based on this table and the ultrasound prostate volume reported by the urologist. Seeds were placed in each needle at 0.5- to 1.0-cm intervals based on the length of prostate tissue implanted along the length of the needle. To decrease urethral doses, a modified peripheral-loading scheme was developed that placed 75% of the activity in the periphery of the prostate. The D90 doses have steadily increased over time because of the increased efficiency of seed placement. This efficiency was gained by means of continual improvement in operator experience, implementation of biplanar ultrasound technology, and adoption of intraoperative computer-based dosimetry (20).

The dose delivered to the prostate was calculated with a 1-month postimplantation computed tomography–based dosimetric analysis. All patients were asked to return 1 month after implantation for computed tomographic scanning. Reasons for not performing dosimetry were poor visualization because of hip prostheses or patient noncompliance. Implant dose was defined as the D90. A median of 92 seeds (range, 31–220 seeds) were implanted. Median activity implanted was 44.6 mCi (range, 7.6–96.9 mCi). The range of D90 values was 180.0–267.3 Gy, with a median of 197.5 Gy. The range of V150 values was 0.0–94.1%, with a median of 69%. Median urethral V150 was 0.08 ml (range, 0.00–2.08 ml), and median rectal V100 was 1.00 ml (range, 0.00–6.19 ml).

Neoadjuvant hormonal therapy with a luteinizing hormone–releasing hormone analogue with or without an antiandrogen was used in 199 patients (31%). Hormonal therapy in conjunction with brachytherapy was used for two main reasons. Hormonal therapy was used for patients with large prostates (gland size > 50 cm^3) or those with intermediate- or high-risk disease. In general, it was given for 3 months before and 3 months after implantation.

Follow-up

All patients were asked to return for follow-up visits every 6 months after completion of treatment. Attempts to obtain follow-up information included mailed questionnaires and telephone surveys. In addition, the final status of a patient was checked with the Social Security Death Index to determine alive/dead status and

date of death. All patients who died during the study period were followed up to determine cause of death and prostate cancer disease status. Follow-up was calculated from completion of treatment to last available follow-up date or date of death. We analyzed biochemical outcomes of 531 patients who had a minimum 2-year PSA follow-up or experienced a PSA failure within the first 2 years (median follow-up, 6.7 years; range, 2.0–11.1 years). A PSA failure was determined using the American Society for Therapeutic Radiology and Oncology (ASTRO) definition and the nadir plus two definition (21, 22).

Erectile function was assessed by physicians (R.G.S., N.N.S.) before and after brachytherapy by using a scoring system of 0, which indicates complete inability to have erections; 1, able to have erections, but insufficient for intercourse; 2, can have erections sufficient for intercourse, but considered suboptimal; and 3, normal erectile function. A score of 2 or 3 (erections sufficient for intercourse) is considered potent. The relevance of this scoring system was described previously (23). Of 572 patients with available potency data, 420 (73.4%) were potent before implantation. To assess longitudinal self-reported urinary function and quality of life by using a validated questionnaire, 249 patients with pretreatment International Prostate Symptom Score (IPSS) and urinary quality-of-life score and a follow-up IPSS at least 3 years after implantation (median, 4.5 years; range, 3–10 years) were analyzed. Routine ultrasound-guided prostate biopsy at 2 years or at the time of PSA failure and/or suspected local recurrence was offered to patients, although compliance was not uniform. A total of 121 patients underwent prostate biopsy 2 or more years after brachytherapy, which were interpreted by a single pathologist. Biopsies were read as positive or negative, with none read as indeterminate. Any residual cancer, even with radiation effect, was counted as positive.

Statistics

Survival curves were determined using the methods of Kaplan and Meier. Differences in survival rates were calculated using the log-rank test. Multivariate analysis of survival and urinary and rectal toxicity was performed using Cox regression analysis. Differences in proportions were tested using the chi-square test.

RESULTS

Biochemical control and biopsy results

Five- and 7-year biochemical disease-free survival (bDFS) rates for the entire cohort using the ASTRO definition were 96.9% and 93.9%, respectively (Fig. 1a). Using the nadir plus two definition, 5- and 7-year bDFS rates for the entire cohort were 96.5% and 91.7%, respectively (Fig. 1b). Five- and 10-year overall survival rates were 96.7% and 90.3%, respectively. For patients with NCCN low-risk disease, the 5-year bDFS rate using the ASTRO definition was 98.2%, whereas for NCCN intermediate/high-risk disease, the 5-year bDFS rate was 91.3% ($p = 0.004$; Fig. 2a). Using the nadir plus two definition, 5-year bDFS rates for low- and intermediate-risk patients were 97.3% and 92.8%, respectively ($p = 0.11$; Fig. 2b). Interestingly, on multivariate analysis, including pretreatment PSA, stage, Gleason score, age, D90 dose, prostate volume, and hormones, only PSA predicted for PSA failure (hazard ratio, 1.13; $p = 0.004$). The biopsy-positive rate at 2 years was 4.1%.

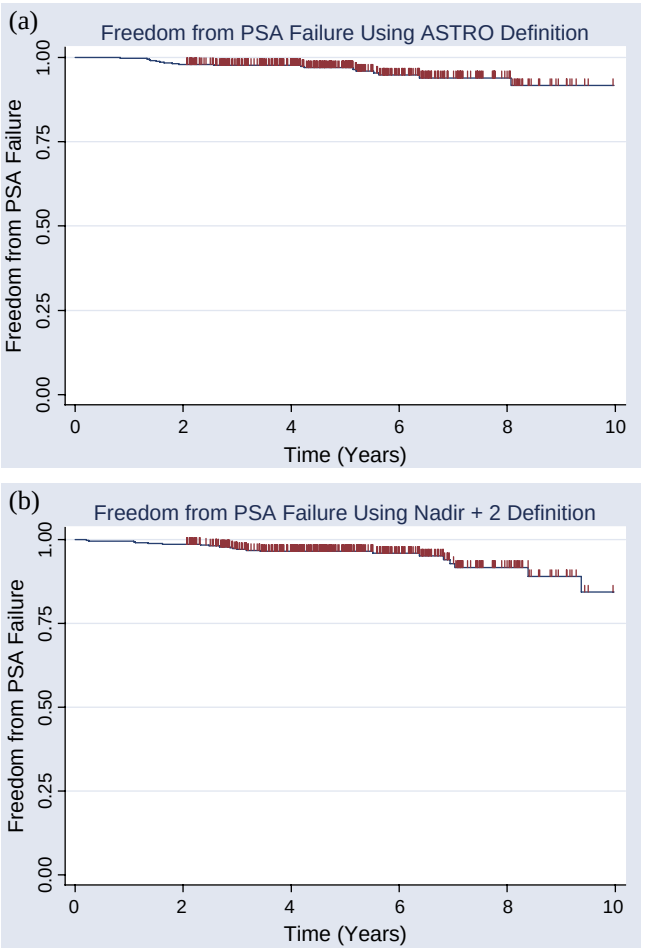

Fig. 1. Five- and 7-year biochemical disease-free survival rates for the entire study population using the (a) American Society for Therapeutic Radiology and Oncology (ASTRO) definition are 96.9% and 93.9% and (b) Phoenix definition are 96.5% and 91.7%, respectively. PSA = prostate-specific antigen.

Acute and late toxicity

Freedom rates from National Cancer Institute Common Terminology Criteria for Adverse Events Grade 2 or higher rectal bleeding at 3 and 5 years were 91.2% and 88.5%, respectively (Fig. 3). Although greater rectal V100 was associated with increased risk of rectal bleeding ($p = 0.009$), prostate D90 failed to predict rectal bleeding ($p = 0.08$). Freedom rates from Grade 2 or higher rectal bleeding at 3 years were 94.1% for patients with a rectal V100 less than 1.3 ml vs. 88.6% for patients with a rectal V100 of 1.3 ml or greater. Acute urinary retention occurred in 12.4% of patients, and the only significant predictor of this event on univariate and multivariate analysis was pretreatment IPSS ($p < 0.01$). Rates of acute urinary retention were 21.1% for a pretreatment IPSS of 20 or higher vs. 12.0% for lower pretreatment IPSSs. Ultrasound prostate volume ($p = 0.07$), use of hormonal therapy ($p = 0.31$), pretreatment transurethral resection of the prostate ($p = 0.81$), urethral D30 ($p = 1.0$), and prostate D90 ($p = 0.48$) failed to predict risk of urinary retention. As expected, median IPSS increased significantly during the first 6 months after implantation (Fig. 4a). Although scores decreased

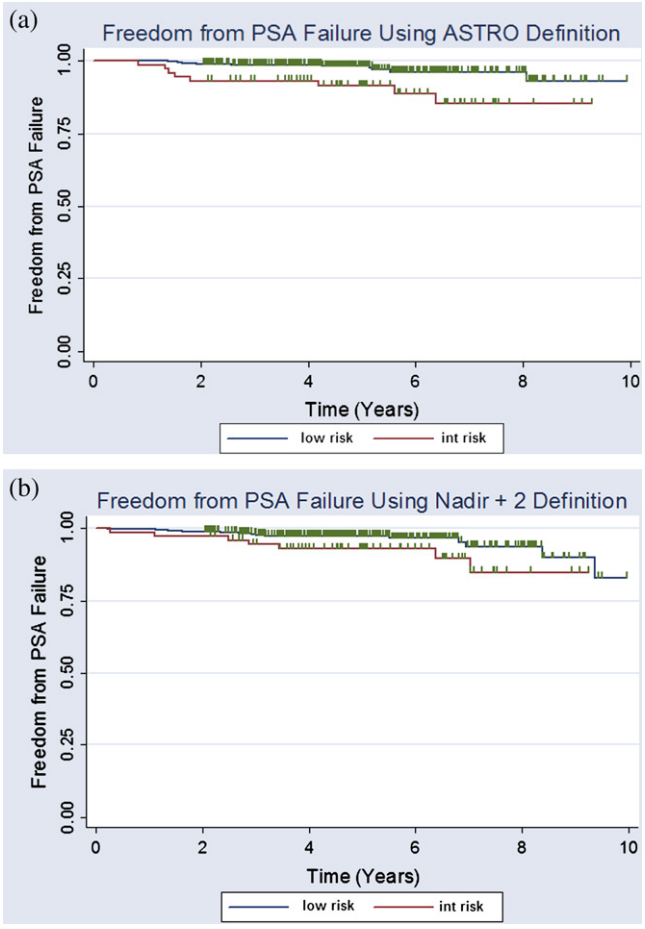

Fig. 2. Five-year biochemical disease-free survival rates stratified for low-risk (blue line) and intermediate-risk patients (red line) using the (a) American Society for Therapeutic Radiology and Oncology (ASTRO) definition are 98.2% and 91.3% ($p = 0.004$) and (b) Phoenix definition are 97.3% and 92.8%, respectively ($p = 0.11$).

significantly from 6 to 24 months, we noted evidence of a late urinary symptom flare from 24 to 30 months. However, there were no numeric or statistical differences comparing

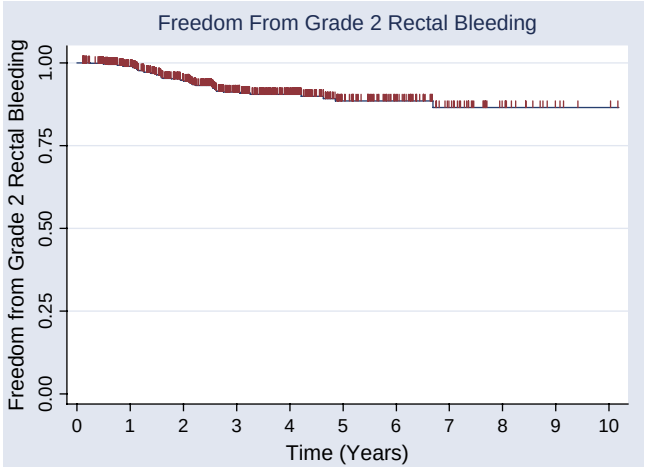

Fig. 3. Three- and 5-year actuarial freedom rates from Grade 2 or higher rectal bleeding for the entire study population are 91.2% and 88.5%, respectively.

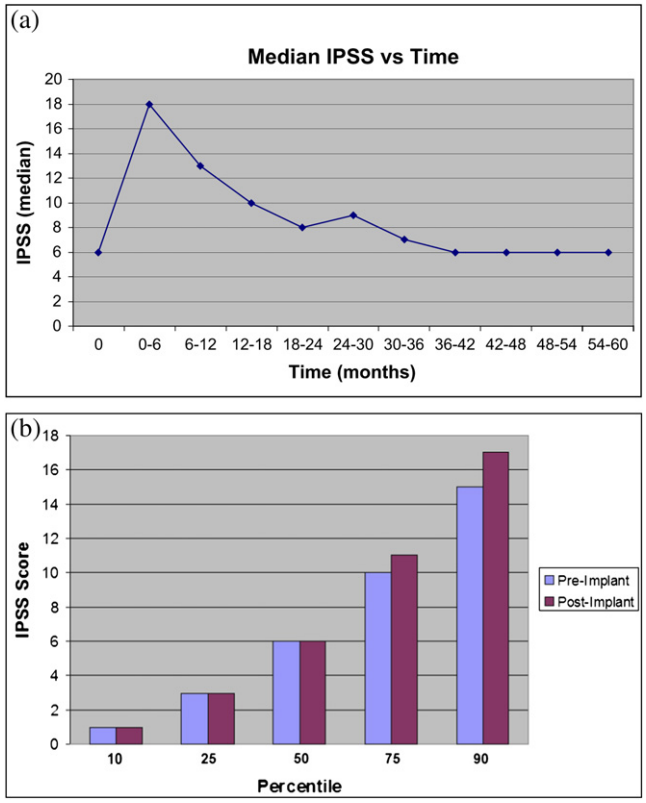

Fig. 4. (a) Change in median International Prostate Symptom Score (IPSS) for the entire study population vs. time; median IPSSs increase significantly during the first 6 months after implantation. (b) Distribution of pre- vs. postimplantation IPSSs.

IPSS before and after implantation with long-term follow-up beyond 3 years ($p = 0.14$). Median IPSSs before and after implantation were 6. Statistical distributions of IPSSs before and after implantation were nearly identical (Fig. 4b; Table 2). There were no detectable differences in patient-reported urinary quality of life before and after implantation ($p = 0.75$). Median urinary quality-of-life scores before and after implantation were 2. In patients who were potent before treatment, 86.8% and 73.4% remained potent at 3 and 5 years after implantation, respectively.

Table 2. Distribution of IPSSs before and after seed implantation in 249 patients with available pre- and posttreatment IPSSs with minimum of 3-year follow-up

	IPSS				
10%	25%	50%	75%	90%	Score
Pretreatment					
1	3	6	10	15	IPSS 0–28
0	0	2	3	4	QOL 0–6
Posttreatment					
1	3	6	11	17	IPSS 0–31
0	1	2	2	3	QOL 0–6

Abbreviations: IPSS = International Prostate Symptom Score; QOL = quality of life.

Median follow up, 4.5 years; range, 3–10 years.

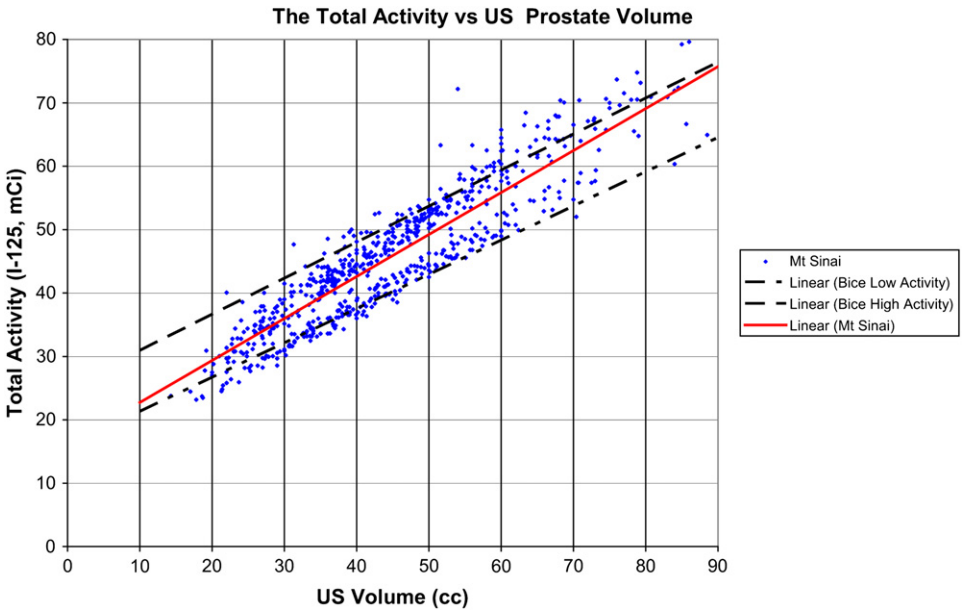

Fig. 5. Total activity vs. ultrasound (US) prostate volume for Mount Sinai and the upper and lower limit of the multi-institutional analysis from Bice *et al.* (26). Dots represent individual patients at Mount Sinai, whereas lines are generated by the method of least squares; the dependent variable is total activity and the independent variable is its corresponding ultrasound volume.

DISCUSSION

Classic modeling of radiation dose vs. tumor control or major complication suggests a sigmoid curve (24). Although the precise dose range of the linear portion of the curve is variable, increasing data for patients with prostate cancer suggest that the probability of tumor control increases significantly at external beam doses greater than 70 Gy and prostate brachytherapy doses greater than 145 Gy (2, 4–8, 25). If greater doses can be delivered to the tumor, greater log-kill of tumor cells is achieved. Assuming a marginal benefit for increased dose, if normal-tissue doses can be kept within tolerance levels an increase in therapeutic ratio is achieved (24).

There are significant technical differences in implantation techniques among major prostate brachytherapy centers (26). Using a real-time ultrasound-guided technique, an experienced brachytherapy team can safely achieve prostate dose escalation with relatively homogeneous implants that result in significant rectal, urethra, and penile bulb sparing (24, 27). In addition to using intraoperative planning, our group does not use extracapsular seeds (28). In 1997, Bice *et al.* (26) showed significant variability among centers in the amount of activity implanted into the prostate (Fig. 5). Because of the accuracy and reproducibility of the real-time technique, although total activity implanted/volume is within the accepted range of clinical practice, the implants described deliver an at least 25% greater dose to the prostate than a standard implant prescribing a D90 of 144 Gy (26). In this context, analyzing both total activity implanted/volume and D90 might provide an important metric of optimizing implant quality.

This group of patients with a minimum D90 of 180 Gy had outstanding PSA control despite the inclusion of some intermediate- and high-risk patients, with a toxicity profile similar to lower dose implants (29). In particular, long-term urinary and sexual function outcomes were excellent. In this study, greater prostate D90 did not predict for acute or late toxicity. Instead, normal tissue toxicity appeared more closely associated with such previously described treatment and patient factors as rectal dose, pretreatment urinary function, and genetic predisposition (27, 30, 31). Because this report is primarily descriptive, we made no attempt to compare biochemical outcome with low (<130 Gy), intermediate (130–144 Gy), high (145–160 Gy), and very high prostate D90s (>160 Gy).

There are a number of weaknesses of this report inherent to a descriptive retrospective study. Although implants were prescribed to 160 Gy by using an intraoperative technique that resulted in relatively high D90s, patients were not selected prospectively to receive doses greater than 180 Gy. Additionally, because of variations in practice patterns over time and among various referring urologists, interventions that included seminal vesicle biopsy before treatment, hormone administration, collection of longitudinal IPSSs, and follow-up prostate biopsies were not carried out in a uniform fashion. Therefore, it is appropriate to interpret this study as a selected subgroup analysis of a larger brachytherapy experience for low- and intermediate-risk patients with prostate cancer.

In conclusion, this shows the feasibility and safety of high D90s (in the context of historically acceptable implanted activity/volume) and provides a starting point for brachytherapists to pursue additional studies to optimize prostate dose to maximize local control while attempting to limit normal tissue toxicity.

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CLINICAL INVESTIGATION **Prostate**

A GENETICALLY DETERMINED DOSE–VOLUME HISTOGRAM PREDICTS FOR RECTAL BLEEDING AMONG PATIENTS TREATED WITH PROSTATE BRACHYTHERAPY

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Purpose: To examine whether possession of genetic alterations in the *ATM* (ataxia telangiectasia) gene is associated with rectal bleeding in a dose-dependent and volume-dependent manner.

Methods and Materials: One hundred eight prostate cancer patients who underwent brachytherapy using either an ^{125}I implant, a ^{103}Pd implant, or the combination of external beam radiotherapy with a ^{103}Pd implant and had a minimum of 1 year follow-up were screened for DNA sequence variations in the 62 coding exons of the *ATM* gene using denaturing high-performance liquid chromatography. Rectal dose was reported as the volume (in cubic centimeters) of rectum receiving the brachytherapy prescription dose. The two-sided Fisher exact test was used to compare differences in proportions.

Results: A significant correlation between the presence of any *ATM* sequence alteration and Grade 1 to 2 proctitis was obtained when the radiation dose to rectal tissue was quantified. Rectal bleeding occurred in 4 of 13 patients (31%) with a variant versus 1 of 23 (4%) without a genetic alteration for patients who had <0.7 cm^3 of rectal tissue receiving the implant prescription dose ($p = 0.05$). Of patients in whom 0.7–1.4 cm^3 of the rectum received the implant prescription, 4 of 11 (36%) with an *ATM* alteration exhibited Grade 1 to 2 proctitis, whereas 1 of 21 (5%) without a variant ($p = 0.04$) developed this radiation-induced late effect.

Conclusions: The possession of genetic variants in the *ATM* gene is associated with the development of radiation-induced proctitis after prostate cancer radiotherapy for patients who receive the full prescription dose to either a low or a moderate volume of rectal tissue.

Genetic predictors, Adverse radiotherapy effects, DVH, Prostate cancer, Brachytherapy.

INTRODUCTION

In the treatment of prostate cancer, the efficacy of the various treatment options is of diminishing importance relative to the side-effect profiles because currently available interventions render similar disease-free survival rates (1–3). Radiation-related side effects are mediated by a number of known patient- and treatment-related factors. Patient-related characteristics including age, performance status, nutritional state, severity of diabetes, peripheral vascular disease, and the functional status of the periprostatic organs before radiotherapy are known to increase the incidence and severity of radiation-related side effects (4–6). Regarding treatment-related factors, total dose and dose rate of radiation given to the pelvis, rectum, bladder, and ejaculatory apparatus are also known to affect the incidence of side effects (7–10). Recent insight into the etiology of radiation-induced side effects was obtained from Radiation Therapy Oncology Group (RTOG) trial 94-06, which reported a difference in the incidence of late RTOG Grade 2 rectal bleeding using 1.8-Gy fractions to 79 Gy and 2.0-Gy fractions to 78 Gy; the lower dose per fraction afforded a 9% incidence, compared with a 33% incidence with the higher dose per fraction (11).

With the advent of newer technologies, there is increased interest in decreasing the incidence of side effects. With intensity-modulated radiotherapy and image-guided

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radiotherapy, dose formalizations used include normal tissue avoidance algorithms, which measurably decrease the incidence of severe normal tissue side effects. On the basis of this initial success, there is increased willingness to allow treatment-planning systems to automatically define the dose given to associated structures using conformal avoidance algorithms, rather than simply administering a uniform dose to the prostate gland and adjacent structures (12–15).

The use of genetic analysis in the formulation of a patient's radiation treatment plan has the potential to further inform the modern practitioner as to the variability and potential severity of side effects observed in prostate cancer. The product encoded by *ATM* plays a central role in mediating the cellular response to radiation-induced DNA damage (16, 17). It has long been known that patients with the rare autosomal recessive genetic syndrome ataxia-telangiectasia are radiosensitive; in addition, there is expression of other clinically evident sequelae of the syndrome, including cerebellar degeneration, ocular telangiectasias, and immunodeficiency (18–20). A pilot study of 37 men treated with low-dose-rate brachytherapy revealed statistically significant differences between the incidence of *ATM* alterations and their correlation with rectal bleeding, erectile dysfunction, and urinary bother (21). The initial study performed was too small to accurately correlate the incidence of the radiation-related side effects to specific doses given to the periprostatic organs. In terms of associating radiation dose–volume effects to late effects, both the onset of erectile dysfunction and chronic urinary bother have very little precedent for a well-quantified relationship. In contrast, late rectal bleeding does lend itself well to a dose–volume analysis in a relatively small series of patients. Snyder *et al.* (22) reported that ^{125}I implant patients who received a rectal dose of 160 Gy to more than 1.3 cm^3 of tissue had a higher incidence of rectal bleeding than patients with a small amount of rectal tissue exposed to 160 Gy. Similar dose–response relationships for brachytherapy rectal dose and late rectal bleeding have been defined by other brachytherapists (23, 24).

The purpose of this study was to examine the relationship between the genetic status of the *ATM* gene, rectal dosimetry, and rectal bleeding, as well as its effect on developing erectile dysfunction.

METHODS AND MATERIALS

Patients

Peripheral blood was obtained from 108 patients seen in routine follow-up after either treatment with a ^{125}I implant, a ^{103}Pd implant, or the combination of external beam radiotherapy with a ^{103}Pd implant for treatment of prostate cancer. Patients were staged according to the 1992 American Joint Committee on Cancer standard and had biopsy-proven prostatic adenocarcinoma (25). Patient and tumor characteristics are outlined in Tables 1 and 2. Prostate brachytherapy was performed either directly or under the direct supervision of one radiation oncologist (R.G.S.) using a transrectal ultrasound–guided approach to visualize the placement of each radioactive source within the prostate gland through a template-guided transperineal needle using the Mick applicator (26). The implant characteristics are outlined in Table 3. The prescription doses for ^{125}I, ^{103}Pd, and a combination implant using ^{103}Pd and external beam radiotherapy (45 Gy) were 160 Gy, 125 Gy, and 100 Gy, respectively (27, 28). Patients returned 4 weeks after the implant for detailed CT-based dosimetric analysis and treatment planning if external beam radiotherapy was given. External beam radiotherapy was given with either five-field intensity-modulated radiotherapy or a six-field conformal three-dimensional plan to a median total dose of 45 Gy. The combination of external beam radiotherapy and brachytherapy has been previously

Table 1. Patient characteristics and baseline urinary, rectal, and erectile function

Characteristic	No. of patients (%)
Age (y), median (range)	64 (46–79)
Coronary artery disease	14 (13)
Hypertension	35 (32)
Stroke	1 (1)
Any history of smoking	37 (34)
Diabetes mellitus	6 (6)
Pretreatment AUA urinary function score	
Good (0–7)	66 (61)
Moderate (8–19)	28 (26)
Severe (20–35)	4 (4)
No value	10 (9)
History of TURP before implant	3 (3)
Preimplant ultrasound prostate volume (cm^3)	
<35	35 (32)
36–50	46 (43)
>50	27 (25)
Erectile function	
3 (optimal)	53 (49)
2 (suboptimal but sufficient)	22 (20)
1 (insufficient)	16 (15)
0 (none)	17 (16)
Ulcerative colitis/Crohn's disease	1 (1)
Hemorrhoids	11 (10)

Abbreviations: AUA = American Urological Association; TURP = transurethral resection of the prostate.

Table 2. Clinical tumor characteristics

Characteristic	No. of patients (%)
PSA (ng/mL)	Median 6.1, range, 0.8–41
≤4	12 (11)
>4–10	84 (78)
>10–41	12 (11)
Gleason score	
5	5 (5)
6	87 (81)
7	13 (12)
8–10	3 (3)
Stage (AJCC 2002)	
T1b	1 (1)
T1c	64 (59)
T2a	22 (20)
T2b	16 (15)
T2c	4 (4)
T recurrent after 70 Gy in 1996	1 (1)

Abbreviations: PSA = prostate-specific antigen; AJCC = American Joint Committee on Cancer.

Table 3. Postimplant dosimetric parameters of all patients

Implant characteristics	Median (range)
I-125 implants (n = 84)	
Total activity (mCi)	42 (24–71)
Needle number	23 (16–32)
Seed number	96 (48–179)
Dose to 90% of the prostate (Gy)	197 (156–239)
Volume of prostate receiving 150% of prescription dose (%)	70 (36–90)
Dose to 30% of the urethra (Gy)	238 (164–419)
Amount of rectum receiving 100% prescription dose (cm^3)	0.9 (0.01–3.56)
Biologic equivalent dose for the total radiation dose (Gy)	210 (164–258)
Pd-103 implants (n = 24)	
Total activity (U)	158.2 (89.8–333.4)
Needle number	21 (16–28)
Seed number	78.5 (47–158)
Dose to 90% of the prostate (Gy)	104.3 (86.9–155.3)
Volume of prostate receiving 150% of prescription dose (%)	80 (65.9–96.7)
Dose to 30% of the urethra (Gy)	126.9 (103.7–204)
Amount of rectum receiving 100% prescription dose (cm^3)	1.5 (0.17–3.04)
Supplemental external radiotherapy dose for 23 patients (Gy)	45 (39.6–70.2)
Biologic equivalent dose for the total radiation dose (Gy)	184 (159–267)

described (29). For this study, a tabular dose–volume histogram was available for the rectum of each patient.

Definition of adverse response

The departmental prostate cancer tissue repository database provided all necessary clinical and dosimetric data for this study. The database is an institutional review board (IRB)-approved prospective collected resource with data from 2456 patients who have undergone prostate brachytherapy at the Mount Sinai Hospital from June 1990 to March 2006. Every patient had a comprehensive medical history taken and physical examination before the brachytherapy procedure. All patients have been offered continual follow-up evaluations at 6-month intervals, which entail a directed history, prostate-specific antigen determination, and physical examination. Acute and late rectal toxicity were graded according to RTOG morbidity criteria (30). Patients who experienced either RTOG Grade 1 or 2 toxicity were considered to have a radiation-related adverse response. Erectile function was physician assessed using the following scoring system: 0 = complete inability to have erections, 1 = able to have erections but insufficient for intercourse, 2 = can have erections sufficient for intercourse but considered suboptimal, and 3 = normal erectile function. The derivation and relevance of this scoring system has been previously described (31, 32). A decrease by 2 points was defined as a decline in erectile function.

For genetic analysis, each patient was approached at the time of follow-up evaluation by a research coordinator who was not involved in the patient's care. An additional IRB-approved consent form was presented to the patient, beyond the one obtained to collect their clinical data, and consent was obtained for this research study.

ATM *exon characterization*

Isolation of DNA from lymphocytes was accomplished using Ficoll separation, as described previously (33). Polymerase chain reaction was used to amplify each of the 62 exons, and short intronic regions flanking each exon, that constitute the coding region of the *ATM* gene, using primers previously described (34). Denaturing high-performance liquid chromatography (DHPLC) analysis was performed on a WAVE Nucleic Acid Fragment Analysis System (Transgenomic, Omaha, NE) using buffer gradient and temperature conditions calculated using WAVEmaker software (version 3.3, Transgenomic) designed for this purpose. Exons with an aberrant DHPLC chromatogram underwent DNA forward and reverse sequencing using a PRISM 377 DNA Sequencer (ABI, Foster City, CA).

Statistical analysis

Analyses were performed using the SigmaStat version 3.1 statistical software package (SPSS, Inc., Chicago, IL). Differences in proportions were derived using the Fisher exact t test and, when appropriate, the chi-square statistic. A p value of ≤ 0.05 was considered to indicate statistical significance. To compare doses between different isotopes and between the implant alone, and with combined implant and external beam radiotherapy, biologic equivalent dose calculations were performed as has been previously described (29).

RESULTS

Fifty-nine *ATM* genetic alterations, representing 25 different variants, were found in the expressed portions of the gene, or within 10 nucleotides of each exon encompassing potential splice sites, in 48 of the 108 patients studied (Table 4). Most of the sequence alterations detected have been previously described (35–37). A brachytherapy-associated rectal dose–volume effect was found for rectal bleeding in this patient population: 10 of 68 patients (15%) who received the prescription dose to 1.4 cm^3 or less of rectal tissue experienced RTOG Grade 1 or 2 late rectal bleeding, whereas 14 of 40 (35%) who received the prescription dose to a larger volume of rectum exhibited rectal bleeding (p = 0.03) (Fig. 1). However, for patients who received the prescription dose to a similar volume of rectum, an association with the possession of an alteration within *ATM* was observed. For patients whose rectum received less than 0.7 cm^3 of the implant prescription dose, rectal bleeding occurred in 4 of 13 (31%) who had a variant in *ATM*. In contrast, 1 of 23 (4%) without an alteration in this gene developed rectal bleeding (p = 0.05). For patients in whom 0.7–1.4 cm^3 of the rectum received the implant prescription, 4 of 11 (36%) with an *ATM* alteration exhibited Grade 1 to 2 proctitis, whereas 1 of 21 (5%) without a variant (p = 0.04) developed this radiation-induced late effect. There was no statistically significant relationship between possession of *ATM* variants and the development of proctitis for patients in whom more than 1.4 cm^3 of the rectum received the prescription dose (Fig. 2).

Twenty-four patients carried missense alterations coding for an amino acid substitution in the ATM protein. No direct association was found on analysis of missense alterations and rectal bleeding. Of 24 patients with missense mutations, 5 (21%) had rectal bleeding, compared with 20 of 84 (24%)

Table 4. *ATM* variants identified in 48 prostate cancer patients

Patient no.	Nucleotide location	Codon	Mutation type	Amino acid change
1–10	5557G→A	1853	M	D→N
11	167T→C	54	S	Y→Y
12	1810C→T	604	M	P→S
13	198A→C	66	S	K→K
14	2038T→C	680	M	F→L
15	2119T→C	707	M	S→L
16	2362A→C	788	M	S→R
17	2685A→G	895	S	L→L
	2614C→T	872	M	P→S
18	3161C→G	1054	S	P→R
19	378T→A	126	M	D→E
	415G→A	139	M	A→T
20	378T→A	126	M	D→E
	1176C→G	392	S	G→G
	IVS7-8insT	N/A	N/A	N/A
21	5557G→A	1853	M	D→N
	IVS38-8T→C	N/A	N/A	N/A
	IVS62+8A→C	N/A	N/A	N/A
22	1810C→T	604	M	P→S
	4388T→G	1463	M	F→C
23	4473C→T	1491	S	F→F
24–26	4578C→T	1526	S	P→P
27	5557G→A	1853	M	D→N
	4578C→T	1526	S	P→P
28	5557G→A	1853	M	D→N
	IVS38-8T→C	N/A	N/A	N/A
29	5557H	1853	M	D→N
	IVS38-8T→C	N/A	N/A	N/A
30	5558A→T	1853	M	D→V
31	5793T→C	1931	S	A→A
32	2572T→C	858	M	D→E
	9200C→G	N/A	N/A	N/A
33–45	IVS62+8A→C	N/A	N/A	N/A
46	735C→T	245	S	V→V
47, 48	IVS38-8T→C	N/A	N/A	N/A

Abbreviations: S = synonymous; M = missense; N/A = not available.

without an *ATM* alteration ($p = 0.97$). When only patients with <1.5 cm^3 exposed to the prescription dose of their radiotherapy were considered for analysis, 3 of 12 (25%) with an *ATM* alteration experiencing rectal bleeding, compared with 7 of 56 (13%) ($p = 0.5$). When the silent intronic alteration IVS62+8A→C was added to the group with missense alterations, a significant association was discovered: 9 of 22 (41%) experienced late rectal bleeding, compared with 4 of 51 (8%) without such mutations in the low rectal dosing range ($p = 0.002$).

A significant decline in erectile function according to missense alteration status was seen among the 68 men who were initially potent before radiotherapy. With a median follow-up of 45 months (range, 12–107 months), 19 of 68 patients (28%) experienced a significant decline in erectile function. Among the 14 men with missense alterations, 7 (50%) had a significant decline in sexual function, compared with 12 of 54 patients (22%) without missense alterations ($p = 0.05$). Of interest, 8 patients experienced a biochemical disease relapse: 1 of 24 (4%) among patients with *ATM* missense alterations and 7 of 84 (8%) without the alterations ($p = 0.8$). The variables known to affect either prostate cancer treatment outcome or late toxicity were evenly distributed among patients with and without an *ATM* sequence alteration (Table 5).

DISCUSSION

When the genetic status of the patients was taken into account, a significant dose–volume effect was found for the incidence of rectal bleeding within this group of 108 patients treated with brachytherapy for prostate cancer. At the low end of rectal radiation dose exposure, a difference was identified for patients who received the radiation prescription dose to between 0.1 cm^3 and 0.7 cm^3 of rectal tissue. This relationship was again seen in the next dose–volume level of 0.7 cm^3 to 1.4 cm^3. For patients who received the prescription

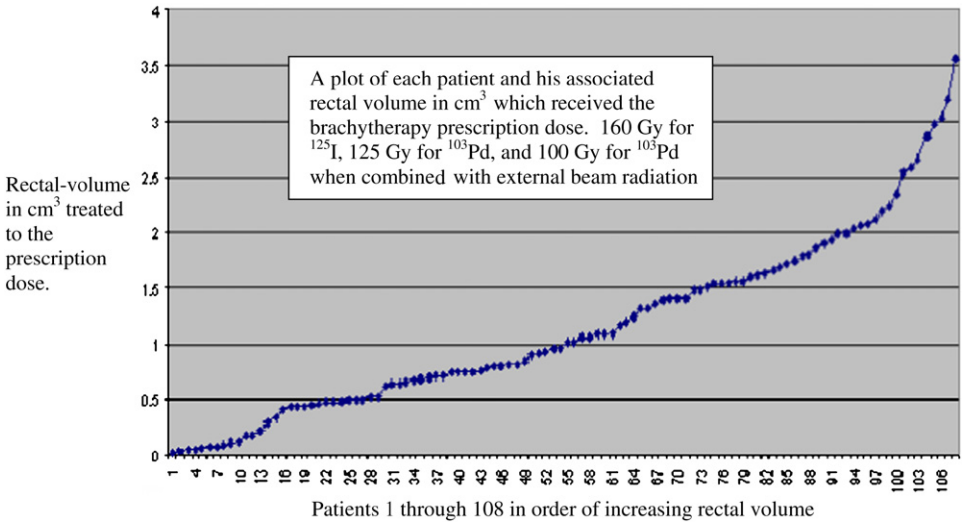
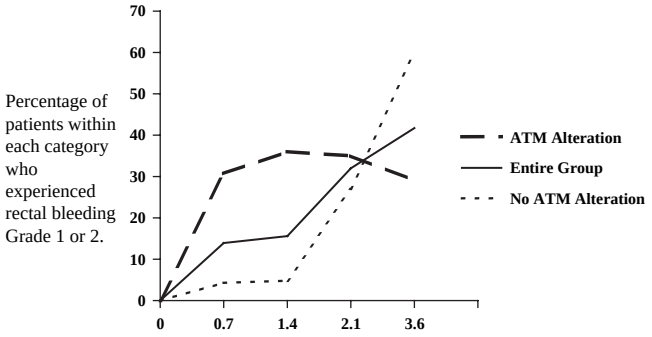


Fig. 1. Rectal dose as represented in volume of rectal tissue treated to the prescription dose using brachytherapy. All 108 patients have a completed characterized *ATM* gene in addition to a detailed clinical history, with a median follow-up of 45 months (range, 12–107 months).



Number of	36	32	28	12
ATM Alteration	4 / 13	4 / 11	6 / 17	2 / 7
No ATM	1 / 23	1 / 21	3 / 11	3 / 5
Fisher's Exact	p=0.05	p=0.04	p=1	p=0.56

Fig. 2. Incidence of Grade 1 or 2 rectal bleeding (%) in the entire group of 108 patients given brachytherapy for prostate cancer and according to their *ATM* gene status.

dose to >1.4 cm^3, there was no longer a difference between the incidence of rectal bleeding among patients with and without an *ATM* variant. On the basis of these results, it is possible to conclude that the slight difference in radiosensitivity conferred by the presence of a variant in *ATM* was of consequence only when a limited volume of rectal tissue was irradiated. Once a threshold in volume receiving the prescription dose had been reached, the genetic status of an individual patient had little effect on the likelihood of them developing rectal bleeding.

The mechanism of injury responsible for late radiation-induced rectal injury occurs mainly in the submucosal region, whereas acute injury is a transient mucosal phenomenon. Late radiation injury is caused by progressive fibrosis, with both the deposition of collagen and fibroblast proliferation noted in the rectal wall. Microvascular injury also occurs and manifests clinically as the appearance of telangiectasias on the rectal surface. If the submucosal region becomes very ischemic, ulceration develops and a fistula can form, although rarely (38–40). In the vast majority of patients, bleeding resolves in a few months without further intervention. In addition, radiation oncologists have lowered the incidence of fistulas by cautioning the care team to avoid biopsy of such regions and to adhere to a conservative observation management policy (41).

In terms of erectile dysfunction, a relationship was found between its development and the presence of *ATM* missense variants. There is some controversy regarding the precise etiology of radiation-induced erectile dysfunction, in that it can either be attributed to the well-known surgical cause, damage to the bilateral neurovascular bundles, or to large-vessel fibrosis and damage (42, 43). It is tempting to hypothesize that the etiology is microvascular in the sense that it is mimicking the apparent mechanism of injury seen in the progression to rectal bleeding. One could hypothesize that a dose–response relationship might become apparent between its onset and the dose to the posterolateral neurovascular bundles if one were able to censure from a studied population those who are genetically predisposed to erectile dysfunction. Because our treatment policy regarding brachytherapy seed insertion does not allow for placement of a radiation source below the urogenital diaphragm, measurable dose to these structures does not occur. This finding will provide for a future investigation informed by the genetic data to identify possible periprostatic radiotherapy targets that might be causally attributed to erectile dysfunction incidence.

When exposed to ionizing radiation, cells arrest their progression through the cell cycle allowing for repair of DNA damage. Much is known about the molecular events that orchestrate the response of the cell to radiotherapy-induced damage. An early event is the formation of the MRE11-RAD50-NBS1 complex, which causes the recruitment of the ATM protein. The ATM protein then acts by interacting with various substrates to halt the cell cycle progression in the G1, S, and G2 phases of the cell cycle. The molecules responsible for these regulatory steps include p53, CHK1, CHK2, MDM2, BRCA1, MDC1, and 53BP1 (44, 45). It is reasonable to hypothesize that if exacerbated normal tissue effects are observed within the radiation dose range that is well tolerated by the majority of patients, a similar magnification of the radiation effect is likely to occur within the sensitive individual's cancer cells. The hypothesis of improved cancer prognosis as a consequence of extreme late radiation normal tissue side effects has been tested in terms of breast cancer without success (46). Prostate cancer is a slow-growing local phenomenon in the majority of patients, with local cure conferring long-term systemic cure (47). This is not the case regarding breast cancer, which since the mid-1970s has been thought to be a systemic disease from onset (48). Though

Table 5. Univariate analysis of the distribution of variables between patients possessing a variant in *ATM* (n = 48) and the patients carrying two wild-type alleles (n = 60) that have been previously described to predict for both toxicity and PSA recurrence

Variable	ATM variant (+)	ATM variant (−)	p
Gleason sum	6 ± 0.4	6 ± 0.8	0.73*
PSA (ng/mL)	7.7 ± 5	7.2 ± 5.8	0.39*
Stage (T1 vs. T2) (n)	26 vs. 22	39 vs. 21	1†
BED (Gy)	207 ± 26	200 ± 25	0.24*
Age (y)	64 ± 8	64 ± 8	0.71‡
Follow-up (mo)	43 ± 22	47.5 ± 22	0.35‡
Hormone therapy	35	45	1†
Addition of EBRT	21	22	1†
Smoker	31	37	1†
Diabetes	2	8	1†
Hypertension	31	33	1†
Coronary artery disease	15	12	1†
African American	15	17	1†

Abbreviations: PSA = prostate-specific antigen; BED = biologic equivalent dose; EBRT = external beam radiotherapy.

Values are mean ± standard deviation or percentage of group, unless otherwise noted.

* Mann-Whitney Rank sum test.

† Fisher exact t test.

‡ Student t test.

radiotherapy does confer a dramatic local control benefit in the setting of breast conservation, only recently has a survival advantage been apparent on the basis of analysis of 42,000 patients with the use of radiotherapy (49). In our series, only 8 patients experienced biochemical relapse: 1 of 24 (4%) among patients with *ATM* missense alterations and 7 of 84 (8%) without alterations. As our patient series continues to mature and increase in size, the initial numeric difference between those who experience a prostate-specific antigen failure and those who do not holds the possibility of a statistical difference appearing in the future.

CONCLUSIONS

The possession of genetic variants in the *ATM* gene is associated with the development of radiation-induced proctitis after prostate cancer radiotherapy for patients who receive the full prescription dose to either a low or a moderate volume of rectal tissue. This finding supports the hypothesis that a genetically determined dose–response relationship is possible and could be used to predict the probability of side effects associated with radiotherapy and serve as a rational basis for individualized radiation dose prescriptions.

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quality of cardiac surgery in that country. In fact, many hospitals in the United States already accept Canadian cardiac surgeons, certified by the Royal College of Physicians and Surgeons of Canada, without any concern for their purported general surgery deficiencies.

There are many benefits to adopting an integrated residency program, both for the residents and the program. For the resident, it would mean a more focused training on cardiothoracic disease, with rotations spent in the cardiac catheterization lab, the echocardiographic lab, in cardiovascular imaging, and rotations in cardiology and cardiac intensive care units. It would give them extra time to pursue focused fellowships in their area of choice (eg, minimally invasive surgery, surgery for arrhythmias, or surgery for heart failure). The resident would finish training not only as a cardiac surgery trainee, but would also have a special niche, allowing the person to flourish in the first years of practice while having a special area of contribution for a new group. For the training programs, guidance of the residents would come earlier, allowing them to mold the skills and exposure they receive. It would also provide them with a larger body to help with education and manpower issues related to resident work-hour requirements. The earlier access to these residents would ensure an ability to train their cardiothoracic skills, which are clearly different than general surgery skill sets. The argument is strong to favor this for 2 to 3 senior years of general surgery training that will be forever lost and unused.

The American Board of Thoracic Surgery agreed in principle to alternative training tracks for thoracic surgeons as long ago as 2001. It is time to implement a new paradigm and begin to offer residents a more complete and efficient training system the first time around.

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MISCELLANEOUS

Overview of the Thoracic Surgery Residents Association

To the Editor:

Since its inception in 1998, the Thoracic Surgery Residents Association (TSRA) has been the official representative organization for thoracic surgery residents in the United States. Each cardiothoracic trainee becomes a member upon enrollment in an Accreditation Council of Graduate Medical Education (ACGME) accredited thoracic surgery residency program and maintains membership until completion of thoracic residency or subsequent advanced fellowship training. The principle mission of the TSRA is to improve thoracic surgery education by providing a forum for the dissemination of academic, administrative, legislative, and clinical practice information to trainees and to facilitate ongoing communication between residents and the Thoracic Surgery Directors Association (TSDA). The TSRA also provides resident representation directly to several important national organizations, including the American Association of Medical Colleges, American Association for Thoracic Surgery (AATS), Accreditation Council of Graduate Medical Education, Joint Commission for Thoracic Surgical Education, Society of Thoracic Surgeons (STS), and CTSNet.

As both trainees and future leaders of our field, residents are acutely aware of several recent trends affecting our specialty. We recognize that the present era of uncertainty will necessarily bring about changes that may affect the structure and focus of resident education and most other aspects of thoracic surgery. By periodically surveying our 350-plus members, the TSRA performs a crucial role in accurately representing the collective view of thoracic surgery residents on a number of issues. It is our hope that information obtained in this manner will provide the leaders of our specialty with additional resources to continually improve the educational process. Furthermore, we believe that a clear appreciation of current resident concerns is critical to understanding and reversing the alarming decline in thoracic surgery residency applications.

In order to provide residents with the opportunity to openly discuss pertinent issues, the TSRA organizes two resident-oriented forums that are held during the annual AATS and STS meetings. These sessions are designed to encourage direct interaction between residents, invited speakers, and representatives of the AATS, STS, and TSDA. The often lively nature of these discussions reflects a genuine interest in residency training shared by the meeting participants and underscores the importance of such dynamic interactions in the course of advanced surgical education.

The importance of identifying true mentors during residency cannot be overstated. Each year, the TSRA formally recognizes individuals who have made outstanding contributions to cardiothoracic surgery education. The Socrates Award is presented to a surgical educator who has demonstrated a significant commitment to excellence in resident education. The Dr Dwight McGoon Award is presented to an individual who has significantly contributed to the clinical and educational development of thoracic surgery residents through inspiring academic and political contributions to the specialty. These awards represent our sincere appreciation for the exemplary contributions of these individuals and we hope that they may serve to further motivate the surgical educators in our field.

We encourage all thoracic surgery residents to actively participate in the TSRA. The timely exchange of information and feedback is essential to help direct the future of our changing specialty. For comments, suggestions, or to learn more about the TSRA, please visit the website: http://www.tsda.org/tsra/tsra_index.htm.

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Adjuvant Radiation With Modern Techniques is the Standard of Care for Stage III Thymoma

To the Editor:

I read with interest the article by Mangi and colleagues [1]. The authors claim that adjuvant radiation is not needed for stage III thymoma based on the retrospective review of

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follow-up records of 45 patients treated between 1972 and 2004 at the Massachusetts General Hospital. Thirty-eight of the patients received radiation based on various criteria including clinical intuition. The comparison group consisted of 7 patients, 5 of whom were observed without adjuvant treatment for more than 12 months. I strongly believe that such a strong negative conclusion regarding efficacy of adjuvant radiotherapy is without merit when based on a very small observation group. In addition, the authors did not consider important quality factors that predict success of radiotherapy.

The irradiated patients were treated with a median dose of 4,550 cGy (range, 3,000 to 6,100 cGy). Of the 38 patients treated with radiation 14 (32%) recurred; of the 5 treated only with surgery and followed-up for more than 12 months, 2 (40%) recurred. Among patients treated with radiotherapy, 10 of 14 (71.4%) recurred in the pleura. Of patients who failed and were observed, 2 of 2 had pleural recurrences (100%).

An interesting article published in 2004 by Zhu and colleagues [2] evaluated disease and treatment-related factors of 175 patients with thymoma of which 41 had stage III disease. Multivariate analysis revealed that the Masaoka stage and radiation dose (50 Gy versus $>$ 50 Gy) were the only factors that predicted survival. Finding a radiation-dose response relationship for survival at a dose level that is higher than the reported median dose of the treated patients in the Massachusetts General Hospital study calls into question the validity of the authors' conclusions. In addition, an article by the same first author in 2002 reported results of 14 patients treated with radiotherapy for stage II thymoma with an identical median radiation dose level of 4,550 cGy (range, 3,000 to 6,100 cGy) and an identical standard error of 188 cGy for the group [3]. The conclusion from the earlier study was that radiotherapy is unnecessary for stage II thymoma, which was based on failure in a single patient in the nonirradiated arm. The same median radiation dose, range, and standard error in two different clinical groups of patients treated at Massachusetts General Hospital with stage II and stage III thymomas is an unlikely statistical coincidence and implies that radiotherapy quality factors were not adequately addressed in either study.

In addition, the authors did not review modern radiation methods in their discussion. These changes will lead to a dramatic decrease in the morbidity of mediastinal radiotherapy [4, 5]. Intensity-modulated radiation therapy and helical tomographic therapy have improved the therapeutic ratio for anterior mediastinal tumors and are being used throughout the country to treat anterior mediastinal tumors.

Based on the data presented, I believe that the authors have failed to make the case that radiotherapy is an unnecessary component in the treatment of stage III thymomas. A central flaw of the article is that it has ignored important radiotherapy quality factors such as dose, field size, target volume, and technique. Also, the side effects of antiquated radiotherapy techniques are overstated and ignore the modern reality of precise radiation treatment planning.

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Reply

To the Editor:

The conclusions we drew from our admittedly rather modest data set of the role of radiation therapy (RT) in stage III thymomas were conservative and meant to be provocative and simulate further studies. We believe these conclusions are still valid. Dr Cesaretti [1] has added yet another reference (Zhu and colleagues [2]) that supports our conclusions rather than his own. I thank Dr Cesaretti for picking up an error in proofreading; indeed the radiation dosimetry was from our stage II patients rather than our stage III patients. The correct dose was an average of 5,040 Gy (range, 25 to 64 Gy) with 20 of 38 receiving 50 Gy or more. There is no prospective data available on a dose response relationship for RT in thymoma. The Zhu and colleagues reference [2] is seriously misquoted, and I urge students of thymoma to review this article when interpreting Dr Cesaretti's letter [1]. The conclusion of the Zhu and colleagues article (in the abstract, body, and concluding paragraph) was that disease stage and complete resection were the only independent factors that predicted survival in thymoma. They specifically stated that radiation was not a factor in survival and that extending the radiation fields did not enhance local control. In stage III patients, the 5-year local control rate was 72% with RT $<$ 50 Gy and 65% with RT $>$ 50 Gy ($p = 0.76$). The authors further concluded that the role of adjuvant RT in thymoma is not well defined, that not all completely resected patients may need RT, and that prospective trials are needed to define the role of RT in thymoma. In our review of several articles, the role of RT as an adjuvant in thymoma is quite open for debate with all but one article suggesting a questionable benefit to RT. In the absence of a prospective trial, we will never know for sure. I believe RT for thymoma as an adjuvant got started in the 1960 to 1970 era when thoracic surgery was still in its nadir with relatively poor resections done by modern standards. Just as RT has become guided by computed tomography, which clearly has better quality, so has thoracic surgery. Most recent reports from high-volume centers find little evidence that RT as an adjuvant is efficacious. In the largest study to date (Kondo and colleagues [3]) with 170 stage III patients, there was no difference in local recurrence or survival with the addition of RT. The famous PORT (Postoperative Radiotherapy) trial [4] documented that there was a decrease in survival in completely resected stage I and II lung cancers with modern RT, confirming that RT is not "free." Radiation therapy is clearly indicated in an incomplete resection and is probably indicated when the margins are very close. It is quite clear from our results and many other centers that when a complete resection is done, prolonged freedom from

Adjuvant Radiotherapy Improves Overall Survival for Patients With Lymph Node-Positive Head and Neck Squamous Cell Carcinoma

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BACKGROUND. Although adjuvant radiotherapy (RT) is often recommended for locally advanced squamous cell carcinoma of the head and neck (HNSCC), its effect on overall or cancer-specific survival has not been clearly demonstrated. In the current study, the frequency and effect of adjuvant RT on overall survival was investigated in patients with resected lymph node-positive head and neck cancer.

METHODS. Within the Surveillance, Epidemiology, and End Results (SEER) database, patients were selected with lymph node-positive HNSCC (American Joint Committee on Cancer and SEER stage 3/4) who were treated either with surgery alone or surgery and RT and were diagnosed between 1988 and 2001. A total of 8795 patients who met the inclusion criteria for analysis comprised the study population, with a median follow-up of 4.3 years for patients still alive at the time of last follow-up.

RESULTS. Adjuvant RT was utilized in 84% of patients. Adjuvant RT improved the 5-year overall survival (43.2% [95% confidence interval (95% CI), 41.9–44.4%] for surgery + RT vs 33.4% [95% CI, 30.7–36.0%] for surgery alone; $P < .001$) and cancer-specific survival (50.9% for surgery + RT vs 42.1% for surgery) on univariate analysis. On multivariate analysis, adjuvant RT (hazards ratio [HR] of 0.78; 95% CI, 0.71–0.86 [$P < .001$]) remained a significant predictor of improved survival. The significant benefit of radiation on overall survival was noted for lymph node-positive patients with both primary tumors localized to the involved organ (HR of 0.81; 95% CI, 0.71–0.94 [$P = .007$]) and more locally invasive primary tumors (HR of 0.77; 95% CI, 0.68–0.87 [$P < .001$]).

CONCLUSIONS. In what to the authors' knowledge is the largest reported analysis of adjuvant RT in patients with locally advanced HNSCC published to date, adjuvant RT resulted in an approximately 10% absolute increase in 5-year cancer-specific survival and overall survival for patients with lymph node-positive HNSCC compared with surgery alone. Despite combined surgery and adjuvant RT, outcomes in this high-risk population remain suboptimal, emphasizing the need for continued investigation of innovative treatment approaches. ***Cancer* 2008;112: 535–43.** © *2007 American Cancer Society.*

KEYWORDS: adjuvant radiotherapy, squamous cell carcinoma, head and neck, survival.

Although approximately 80% to 90% of patients with stage I or II head and neck cancer are cured with surgery or radiotherapy (RT) alone, outcomes for patients with locally advanced stage III to IVB head and neck cancer have been less promising.[1–3] Because the primary pattern of failure is locoregional, combined surgery and RT has been advocated to address disease above the clavicles in a comprehensive fashion.[4,5] Although this approach has been widely prac-

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ticed since Maccomb and Fletcher's landmark article in 1957,[6] whether surgery and RT improves survival compared with surgery alone has not been definitively proven. Although 2 small randomized trials suggested an improvement in locoregional control for combined surgery and RT compared with surgery alone, neither was adequately powered to demonstrate an overall survival advantage.[7,8]

A matched pair analysis performed at the Mayo Clinic demonstrated improved neck control, cause-specific survival, and overall survival for patients with stage III to IV head and neck cancer who were treated with combined surgery and RT compared with surgery alone.[9] Additional retrospective comparative series from the University of Texas M. D. Anderson Cancer Center, the University of Florida, and the Medical College of Virginia demonstrated significantly improved locoregional control with the addition of adjuvant RT to surgery.[5,10,11] RT may improve locoregional control via 3 mechanisms: improving local control at the primary tumor site, improving regional control of a dissected neck, or sterilizing occult disease in the undissected neck. Multiple randomized trials have now demonstrated that improved locoregional control translates into a survival advantage, even if there is no detectable impact on distant metastases.[12–14] Adjuvant RT is currently indicated for advanced lymph node disease (N2-3); extracapsular extension; close or positive surgical margins; bone, perineural, or lymphovascular invasion; and high likelihood of occult disease in an undissected neck.[2,15] It is unlikely that a large randomized trial will ever be mounted to definitively demonstrate the impact of adjuvant RT on survival for advanced head and neck cancer. Therefore, a retrospective review of high-quality, population-based data would provide significant insight into the impact of adjuvant RT on cancer-specific and overall survival.

MATERIALS AND METHODS

Data and Study Population

The Surveillance, Epidemiology, and End Results (SEER) database is a longitudinal database that collects information from 17 cancer registries covering 26% of the U.S. population.[16] The SEER database is composed of 17 population-based cancer registries from Connecticut, New Jersey, Atlanta, Kentucky, Louisiana, Rural Georgia, Detroit, Iowa, Hawaii, New Mexico, Seattle-Puget Sound, Utah, San Francisco-Oakland, San Jose-Monterey, Los Angeles, Greater California, and Alaska Native Tumor Registry.[17] Serial registry data are deidentified and submitted to the U.S. National Cancer Institute on a biannual basis and these data are publicly available for researchers.[17] Therefore, approval by an ethics committee is not necessary to perform the analyses.[18] The population covered by the SEER database is considered representative of the U.S. population and the case ascertainment rate is reportedly 97.5%.[18]

The histologic types selected for analysis are those coded as squamous cell carcinoma or variants of squamous cell carcinoma (papillary squamous cell carcinoma, verruccous carcinoma, squamous cell carcinoma, lymphoepithelial carcinoma, adenosquamous carcinoma, and basaloid squamous carcinoma) based on the International Classification of Diseases of Oncology codes (ICD-O-2).[19] We identified adult patients aged $\geq$21 years with pathologically confirmed squamous cell carcinoma of the head and neck (HNSCC) (coded as lip, oral cavity, oropharynx, hypopharynx, larynx, sinonasal and middle ear, salivary gland or other oral cavity, and pharynx) diagnosed between 1988 and 2001 who were treated with cancer-directed surgery (n = 42,076). We excluded patients with nasopharyngeal cancer because the primary locoregional treatment is RT rather than surgery. Patients with metastatic disease or tumors of an unknown stage (n = 5586), in situ carcinoma (n = 2183), no pathologic confirmation (n = 1), and unknown administration of RT (n = 842) were also excluded. Use of RT was abstracted by local tumor registries and reported to SEER. Patients were considered to have received RT if they received external beam RT, brachytherapy, or both. Patients who received only radioisotopes (n = 9) were not considered to have received adjuvant RT. To account for perioperative mortality, 516 patients were excluded who died within 4 months of diagnosis. A total of 24,153 eligible patients were lymph node negative. The remaining 8795 lymph node-positive patients were included for this analysis. The most recent follow-up available was November 2005 and the median follow-up available for living patients was 4.7 years.

Statistical Analysis

Categoric variables included patient age at diagnosis ($<$50 years, 50–69 years, or $\geq$70 years), sex, race, year of diagnosis, primary site, SEER 1977 stage (localized or invasive), lymph node stage (N1, N2a, N2b, N2c, N3, or supraclavicular lymph nodes), lymph node surgery, tumor size ($\leq$2 cm, 2.1–4 cm, and $\geq$4 cm), tumor grade, marital status, and use of RT. Marital status has recently been shown to be an important prognostic factor for head and neck cancer patients.[20] Due to the prognostic significance of primary tumor extent in head and neck cancer, we specifically analyzed the effect of RT on survival on

TABLE 1
SEER 1977 Summary Staging System[34]

Stage	Description
0	In situ: noninvasive; intraepithelial
1	Localized only: invasive tumor confined to primary site
2	Regional by direct extension only: invasive tumor extending to adjacent organs and/or subsites
3	Regional lymph node(s) involved only
4	Regional by both direct extension and regional lymph node(s)
7	Distant site(s)/lymph node(s) involved
9	Unknown

SEER indicates the Surveillance, Epidemiology, and End Results program.

localized primary tumors and more extensive primary tumors. Although the American Joint Committee on Cancer (AJCC) T classification was not available in the SEER database, SEER 1977 provided a measure of primary tumor extent (Table 1). SEER stage 3 is defined as lymph node-positive patients with a primary tumor localized to the involved site without extension to adjacent organs or subsites. By contrast, SEER stage 4 includes patients with positive lymph nodes and a primary tumor that extends to adjacent organs or subsites.

Patient age in years, tumor size, and year of diagnosis were analyzed as categoric variables on univariate analysis but as continuous variables on multivariate analysis. Information regarding surgical margin status, extent of lymph node surgery, extracapsular extension, perineural or lymphovascular invasion, use of adjuvant chemotherapy, performance status, recurrent or second head and neck primary tumor, and RT details (dose, fractionation, 3-dimensional conformal/intensity modulated RT, etc.) were not available within the SEER database and this information is not included for analysis. Overall survival was the primary endpoint and cancer-specific survival was the secondary endpoint. To determine the effect of adjuvant RT survival stratified by primary tumor invasiveness and extent, subset analyses were performed on patients with SEER stage 3 and 4 disease.

The Pearson chi-square test was utilized to assess unadjusted associations between adjuvant RT and categoric variables. Overall survival was calculated from the time of diagnosis to the time of death or last follow-up. Cancer-specific survival was calculated from the time of diagnosis to the time of death from any cancer or last follow-up. Nonparametric survival estimates were calculated by the Kaplan-Meier method (product-limit estimate). When applicable, the stratified log-rank test was utilized to compute survival estimates were within specified strata levels. Cancer-specific survival was calculated using SEER*Stat software (version 6.2.4). All other data were analyzed using Stata software (version 8.0; StataCorp, College Station, Tex) by importing data from the SEER (available at URL: www.seer.cancer.gov accessed on November 21, 2006) 1973–2003 Public Use Data (National Cancer Institute, April 2006 release based on the November 2005 submission) into Stata. Results were considered to be statistically significant when $P < .05$.

Cox proportional hazards regression modeling was limited to covariates that we found to be statistically significant on univariate analysis. Due to missing data, a multivariate analysis was developed for the 4572 patients with complete datasets. A multivariable Cox model was developed to calculate the adjusted hazards ratios (HRs) and 95% confidence intervals (95% CIs). Separate multivariate models were developed for 3 groups: all lymph node-positive patients, patients with localized tumors but positive lymph nodes (SEER stage 3), and patients with invasive tumors and positive lymph nodes (SEER stage 4). A formal examination of the proportional hazards assumption was performed graphically by plotting –log(log(S(t)) versus log(t) for each covariate. This confirmed that the covariates are independent with respect to time and their HRs are constant over the clinically relevant period of follow-up.

RESULTS

Among the 8795 patients with lymph node-positive HNSCC meeting eligibility criteria, 7379 (84%) received adjuvant RT. Nearly 96% of irradiated patients received external beam RT alone with 3% receiving external beam RT and brachytherapy, 0.3% receiving brachytherapy alone, and 0.7% receiving an unknown method of RT. Nearly 89% of irradiated patients received postoperative RT, whereas 7% received preoperative RT, 1% received both preoperative and postoperative RT, 0.2% received intraoperative RT with or without additional RT, and in 3% of patients the sequence of surgery and RT was unknown. The median patient age at diagnosis was 60 years (range, 21–100 years). A description of patient demographics and tumor characteristics and their relation to adjuvant RT use is shown in Table 2. Strong predictors of RT use were younger patient age, male sex, non-Black race, diagnosis after 1992, locally invasive tumor, nonoral cavity or salivary gland primary tumor, advanced lymph node disease, first primary tumor, and single or married marital status. By contrast, tumor size failed to predict for adjuvant RT use.

TABLE 2
Patient Characteristics and Prevalence of Adjuvant RT Use

Demographic	No of patients (n = 8795)	% Who received observation (n = 1416)	% Who received adjuvant RT (n = 7379)	*P*
Age, y				<.001
<50	1958	13.0	87.0	
50–69	4869	14.3	85.7	
≥70	1968	23.6	76.4	
Sex				<.001
Male	6588	14.8	85.2	
Female	2207	20.0	80.0	
Race				.016
White	7317	16.1	83.9	
Black	1061	18.3	81.7	
Asian/Pacific Islander/ Native American	408	11.0	89.0	
Other	3	33.3	66.7	
Unknown	6	16.7	83.3	
Year of diagnosis				.001
1988	412	23.1	76.9	
1989	379	19.8	80.2	
1990	389	18.3	81.8	
1991	358	14.3	85.8	
1992	552	20.1	79.9	
1993	559	15.0	85.0	
1994	565	12.6	87.4	
1995	509	14.9	85.1	
1996	577	15.6	84.4	
1997	607	15.8	84.2	
1998	645	15.7	84.3	
1999	644	15.7	84.3	
2000	1270	14.7	85.3	
2001	1329	15.6	84.4	
Tumor size, cm				.490
≤2	1856	17.0	83.0	
2.1–4	3350	16.3	83.7	
≥4	1340	15.8	84.2	
Unknown	2249	15.3	84.8	
Tumor extent				.004
Localized (SEER stage 3)	4345	17.2	82.8	
Invasive (SEER stage 4)	4450	15.0	85.0	
N classification (2002 AJCC)				<.001
N1	2736	21.4	78.7	
N2a	1454	16.2	83.8	
N2b	950	14.3	85.7	
N2c	377	16.2	83.8	
N3	268	9.0	91.0	
Other lymph nodes	980	11.2	88.8	
N+ NOS	2030	13.1	86.9	
Primary site				<.001
Lip	61	29.5	70.5	
Other oral cavity	2300	22.1	77.9	
Oropharynx	3412	11.6	88.4	
Hypopharynx	1067	13.1	86.9	
Larynx	1297	17.4	82.6	
Sinonasal and ear	63	14.3	85.7	
Salivary gland	288	26.7	73.3	
Other	307	14.0	86.0	
Grade	610	20.5	79.5	<.001
1 (well-differentiated)	3875	17.7	82.3	
2 (moderately differentiated)	3482	13.7	86.3	
3 (poorly differentiated)	123	18.7	81.3	
4 (undifferentiated)	705	15.2	84.8	
Unknown				.001
Marital status	1278	14.6	85.4	
Single	2281	18.4	81.6	
Widowed, divorced or separated	4931	15.2	84.8	
Married	305	20.3	79.7	
Unknown				

RT indicates radiation therapy; SEER, Surveillance, Epidemiology, and End Results program; AJCC, American Joint Committee on Cancer; NOS, not otherwise specified.

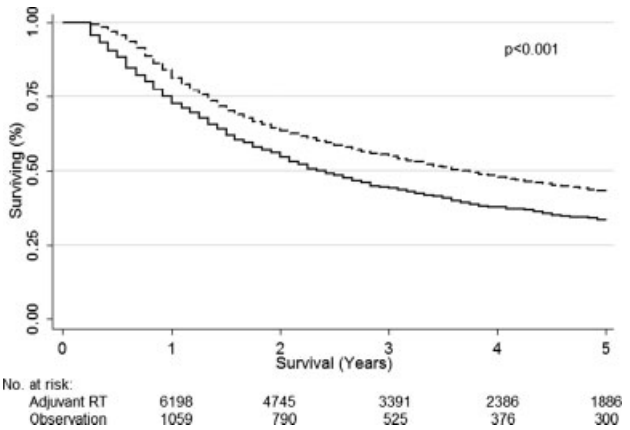

FIGURE 1. Plot of overall survival for all lymph node-positive patients stratified by use of adjuvant radiotherapy (RT). The solid line represents patients receiving surgery alone and the dashed line represents patients who received surgery and RT.

On univariate analysis for all lymph node-positive patients, adjuvant RT was associated with significantly improved overall survival. The 3-year overall survival rate with surgery and RT was 54.9% (95% CI, 53.8–56.1%) compared with 44.4% (95% CI, 41.7–47.0%) for surgery alone ($P < .001$). The 5-year overall survival with surgery and RT was 43.2% (95% CI, 41.9–44.4%) compared with 33.4% (95% CI, 30.7–36.0%) for surgery alone. (See Fig. 1 for Kaplan-Meier plots of overall survival stratified by RT use.) In the largest subset of patients treated by postoperative external beam RT, the 3-year and 5-year overall survival rates were 54.8% (95% CI, 53.5–56.0%) and 43.0% (95% CI, 41.6–

TABLE 3
Univariate Estimates for 3-Year and 5-Year Overall Survival

Demographic	3-Year overall survival	5-Year overall survival	*P*
RT			<.001
Adjuvant RT	54.9	43.2	
Observation	44.4	33.4	
Age, y			<.001
<50	63.7	55.6	
50–69	54.9	42.2	
≥70	38.6	26.5	
Sex			.071
Male	53.9	41.9	
Female	51.3	40.5	
Race			<.001
White	55.2	43.4	
Black	39.7	28.2	
Asian/Pacific Islander/ Native American	52.4	43.4	
Other	33.3		
Unknown	83.3	66.7	
Year of diagnosis			<.001
1988	43.5	31.6	
1989	49.5	35.7	
1990	47.3	34.5	
1991	49.0	34.7	
1992	47.6	37.5	
1993	49.6	37.2	
1994	54.9	43.2	
1995	50.6	40.8	
1996	52.5	42.6	
1997	52.8	42.4	
1998	55.6	44.7	
1999	54.6	45.0	
2000	57.4		
2001	60.4		
Tumor size, cm			<.001
≤2	62.5	50.3	
2.1–4	51.6	39.9	
≥4	40.1	29.5	
Unknown	55.9	44.2	
Tumor extent			<.001
Localized (SEER stage 3)	61.8	49.7	
Invasive (SEER stage 4)	44.9	33.8	
N classification (2002 AJCC)			<.001
N1	62.0	50.5	
N2a	53.3	40.8	
N2b	51.0	39.4	
N2c	46.2	33.3	
N3	38.0	30.4	
Other lymph nodes	48.3	35.8	
N+ NOS	48.0	36.8	
Primary site			<.0001
Lip	41.7	23.7	
Other oral cavity	40.0	30.5	
Oropharynx	68.2	57.5	
Hypopharynx	45.1	31.6	
Larynx	49.6	35.4	
Sinonasal and ear	28.5	18.8	
Salivary gland	46.3	33.5	
Other			
Grade			<.001
1 (well-differentiated)	49.2	38.7	
2 (moderately differentiated)	50.8	39.3	
3 (poorly differentiated)	56.1	44.8	
4 (undifferentiated)	58.9	50.0	
Unknown	54.7	38.9	
Marital status			<.001
Single	47.7	39.1	
Widowed, divorced, or separated	44.9	32.0	
Married	58.2	46.2	
Unknown	57.4	50.2	

RT indicates radiation therapy; SEER, Surveillance, Epidemiology, and End Results program; AJCC, American Joint Committee on Cancer; NOS, not otherwise specified.

44.3%), respectively. Other factors found to be predictive of improved survival were younger age, white or Asian race, diagnosis after 1993, small tumor size, poorly differentiated tumor, lower AJCC 2002 N classification, localized tumor, first primary tumor, and currently married status. Sex failed to significantly impact survival. The results of all univariate analyses of demographics and tumor characteristics and their correlation with overall survival are listed in Table 3. The 5-year cancer-specific survival was 50.9% (standard error [SE]: 0.6%) for surgery and RT versus 42.1% for surgery alone (SE: 1.3%).

To determine whether a measure of tumor invasiveness impacted the efficacy of adjuvant RT, subset analyses were performed for SEER 1977 stage 3 (primary tumor localized to involved site and lymph node positive) and SEER 1977 stage 4 (primary tumor that extends to adjacent organs or subsites and lymph node positive) disease. Additional subset analyses were considered but some patients had missing tumor size and AJCC 2002 N classification. The 5-year overall survival for patients with localized tumors treated with surgery and adjuvant RT was 51.6% (95% CI, 49.8–53.4%) versus 40.6% (95% CI, 36.7–44.5%) for surgery alone ($P < .001$) (see Figure 2 for Kaplan-Meier survival curves for this subset stratified by RT use.) The 5-year cancer-specific survival was 59.9% (SE: 0.9%) with surgery and RT compared with 51.0% (SE: 1.8%) for surgery alone. The 5-year overall survival for invasive tumors treated with combined surgery and RT was 35.3% (95% CI, 33.6–36.9%) versus 25.2% (95% CI, 21.8–28.8%) for surgery alone ($P < .001$). Kaplan-Meier plots for this subset of

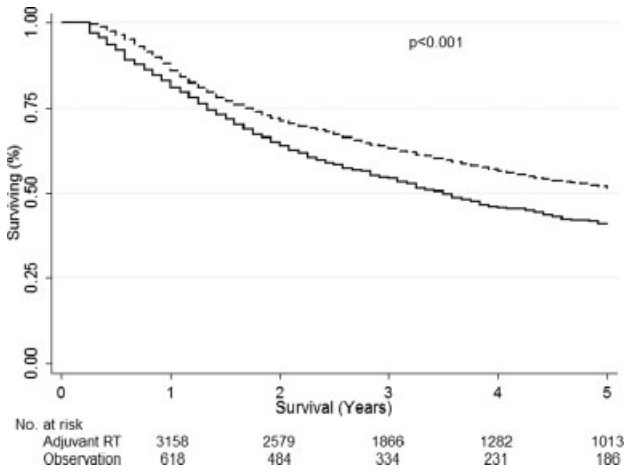

FIGURE 2. Plot of overall survival for patients with lymph node-positive disease and localized primary tumors (Surveillance, Epidemiology, and End Results [SEER] stage 3) stratified by use of adjuvant radiotherapy (RT). The solid line represents patients receiving surgery alone and the dashed line represents patients who received surgery and RT.

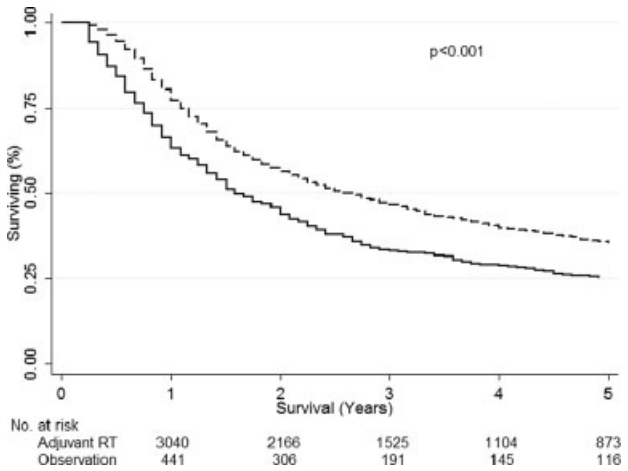

FIGURE 3. Plot of overall survival for patients with lymph node-positive disease and locally invasive primary tumors (Surveillance, Epidemiology, and End Results [SEER] stage 4) stratified by use of adjuvant radiotherapy (RT). The solid line represents patients receiving surgery alone and the dashed line represents patients who received surgery and RT.

patients stratified by RT use are presented in Figure 3. The 5-year cancer-specific survival was 42.3% (SE: 0.8%) for surgery and adjuvant RT and 32.6% (SE: 1.7%) for surgery without RT.

On multivariate analysis of all patients with a complete dataset, adjuvant RT (HR of 0.78; 95% CI, 0.71–0.86 [$P < .001$]), age, primary tumor site, lymph node staging, SEER tumor staging, tumor size, marital status, and race were all found to be significant predictors of overall survival. Year of diagnosis and tumor grade did not significantly improve survival. Separate multivariate analyses for patients with localized tumors and locally invasive tumors demonstrated that the use of adjuvant RT was associated with significantly improved survival in both subgroups. The magnitude of the risk reduction of death was greater for locally invasive tumors (HR of 0.77; 95% CI, 0.68–0.87 [$P < .001$]) than localized tumors (HR of 0.81; 95% CI, 0.71–0.94 [$P = .007$]), but both were found to be statistically significant. The subset analysis recapitulated the findings of the multivariate analysis for all lymph node-positive patients, with the exception that race failed to predict survival for localized tumors and patients with poorly differentiated tumors with locally invasive tumors had better survival. The results of all multivariate analyses are shown in Table 4.

DISCUSSION

The current study was performed to assess the effect of adjuvant RT on cancer-specific and overall survival for patients with locally advanced head and neck cancer. RT was found to significantly improve overall and cancer-specific survival for patients with lymph node-positive, stage III to IVB head and neck cancer. To our knowledge, as the largest reported population analysis of the use of adjuvant RT in patients with locally advanced head and neck cancer published to date, it is significant that our study reveals a clinically significant survival benefit for adjuvant RT in patients with stage III to IVB disease. This information confirms the results of smaller series that demonstrate that adjuvant RT increases cause-specific and overall survival for patients with lymph node-positive head and neck cancer.[2,9,11,21] These data support current guidelines that recommend adjuvant RT for the vast majority of lymph node-positive patients treated with primary surgery.[22]

Although to our knowledge the current study represents the largest published series focused on advanced head and neck cancer patients treated with primary surgery, this U.S. population-based study has a number of limitations that must be considered. The SEER data are collected retrospectively and confounding factors that may have influenced the treating physician's decision to recommend adjuvant RT such as surgical margin status, extracapsular extension, perineural invasion, lymphovascular invasion, and performance status were not available for analysis.[5] Although the presence or absence of lymph node positivity is well documented, N classification was available for 77% of patients, tumor size was documented for 74% of patients, and tumor grade was known for 92%. Whether lymph node surgery

TABLE 4
Predictors of Death From Any Cause in Multivariable Analysis

	All patients (N = 4573)			Localized (N = 2188)			Invasive (N = 2385)		
Demographic (Variable)	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
RT (yes)	.784	.038	<.001	.812	.059	.004	.769	.050	<.001
Age (continuous)	1.022	.002	<.001	1.025	.003	<.001	1.021	.002	<.001
Race (non-black)	.769	.043	<.001	.882	.088	.211	.730	.050	<.001
Site (OPX)	.575	.026	<.001	.481	.035	<.001	.649	.038	<.001
Grade (continuous)	.948	.028	.069	1.028	.046	.539	.896	.035	.005
Year of diagnosis (continuous)	.991	.005	.069	.989	.008	.149	.994	.006	.385
N classification (continuous)	1.126	.014	<.001	1.139	.0228	<.001	1.117	.018	<.001
T classification (invasive)	1.306	.052	<.001						
Tumor size (continuous)	1.007	.001	<.001	1.010	.002	<.001	1.007	.001	<.001
Marital status (continuous)	.885	.023	<.001	.881	.037	.002	.891	.030	.001

HR indicates hazards ratio; 95% CI, 95% confidence interval; RT, radiation therapy; OPX, oropharynx.

was performed was poorly characterized in the database. Specifics of RT quality including dose, field sizes, treatment time, and compliance with therapy are not available. AJCC T classification was not documented for this cohort of patients. SEER T classification for head and neck cancer specifies only in situ, localized, and locally extensive tumors, which does not necessarily correlate with the AJCC T classification. In addition, the database did not contain specific information regarding performance status. The limitations of this database complicate proper interpretation of the data and reduce the power of subset analyses designed to determine cohorts of patients more likely to benefit from adjuvant RT.

To enhance the statistical power of the study, patients with squamous cell carcinoma of all sites, excluding the nasopharynx, were grouped together rather than analyzed separately. Certain subsites appear to have different prognoses and this potentially confounds analysis of the data.[15] Conversely, clinical trials studying advanced head and neck cancer often are not site specific because the prognosis of patients with stage III to IVb head and neck cancer is generally considered poor.[12–14,23,24] Finally, the SEER database did not collect information concerning the use of chemotherapy in this patient population. The additional benefit of concurrent chemotherapy on a subset of patients receiving adjuvant RT was published in 2004.[12,24] Before these studies the use of adjuvant chemoradiation was not considered effective at improving locoregional control or overall survival and this variable is unlikely to be a significant confounding factor.[25,26]

Despite these significant limitations, the HRs for survival for adjuvant RT were found to be greater on multivariate analysis than on univariate variable analysis. These data are consistent with the notion that higher-risk patients within each SEER stage are referred for adjuvant RT and this treatment has a favorable impact on the natural history of advanced head and neck cancer. Conversely, only 16% of the 8795 patients failed to receive adjuvant RT. This raises the possibility that adjuvant RT was considered the standard of care for most patients with lymph node-positive disease and was not offered to less robust patients. We attempted to control for this by excluding patients that died within 4 months of surgery and by incorporating available demographic data into our multivariate analysis. Although adjuvant RT clearly reduced the incidence of death from cancer (absolute benefit of 9.7% at 5 years) and death from any cause (absolute benefit of 9.8% at 5 years), these data do not prove a causal relation. The SEER database does not collect cause of treatment failure, whether locoregional or distant. Taken together, these data support the notion that the benefit attributable to adjuvant RT was not due to imbalances in patient factors favoring the treated cohort.

Although adjuvant RT is associated with significantly improved survival, the outcomes for lymph node-positive patients remain suboptimal. Even with combined surgery and RT, the 5-year survival for patients with lymph node-positive head and neck cancer was only 43%. The vast majority of the deaths were due to cancer. These data are consistent with the surgery + RT arms from the Radiation Therapy Oncology Group (RTOG) and European Organization for Research and Treatment of Cancer (EORTC) trials, which reported 5-year overall survival rates of approximately 40%.[12,24] Taken together, it appears that surgery alone cures approximately one-third of patients with locally advanced but resectable disease.

Adjuvant RT confers an absolute survival benefit of approximately 10%. Particularly for patients with extracapsular extension or microscopic positive surgical margins, adding platinum-based chemotherapy to adjuvant RT further increases survival by 6% to 13%.[27] These data highlight the importance of investigating novel strategies such as increasing RT dose intensity, intensified chemoradiation protocols, and integrating new treatment modalities such as biologic therapy and intensity-modulated RT.[1,15,28–33] Conversely, the finding that marital status affects outcome on both univariate and multivariate analysis suggests that family support might enhance the patient's ability to complete surgery and RT in a timely fashion. These data suggest a potential role for social support services and patient counseling in nonmarried patients, which have become more important in patients receiving intensive combinations of surgery, RT, and chemotherapy. Finally, the data reported herein represent results in a contemporary cohort of patients with locally advanced head and neck cancer who were treated with surgery and adjuvant RT that can be used to compare the efficacy of alternative approaches.

In summary, analysis of the SEER database demonstrated that adjuvant RT offers a significant survival benefit for patients with lymph node-positive squamous cell carcinoma of the head and neck. Future studies are needed to determine whether there are subgroups of patients with lymph node-positive disease that do not benefit from adjuvant RT and whether adjuvant RT improves survival in a subset of patients with locally advanced but lymph node-negative disease.

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Association of Single Nucleotide Polymorphisms in *SOD2, XRCC1* and *XRCC3* with Susceptibility for the Development of Adverse Effects Resulting from Radiotherapy for Prostate Cancer

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The objective of this study was to determine whether an association exists between certain single nucleotide polymorphisms (SNPs), which have previously been linked with adverse normal tissue effects resulting from radiotherapy, and the development of radiation injury resulting from radiotherapy for prostate cancer. A total of 135 consecutive patients with clinically localized prostate cancer and a minimum of 1 year of follow-up who had been treated with radiation therapy, either brachytherapy alone or in combination with external-beam radiotherapy, with or without hormone therapy, were genotyped for SNPs in *SOD2, XRCC1* and *XRCC3*. Three common late tissue toxicities were investigated: late rectal bleeding, urinary morbidity, and erectile dysfunction. Patients with the *XRCC1* rs25489 G/A (Arg280His) genotype were more likely to develop erectile dysfunction after irradiation than patients who had the G/G genotype (67% compared to 24%; $P = 0.048$). In addition, patients who had the *SOD2* rs4880 T/C (Val16Ala) genotype exhibited a significant increase in grade 2 late rectal bleeding compared to patients who had either the C/C or T/T genotype for this SNP (8% compared to 0%; $P = 0.02$). Finally, patients with the combination of the *SOD2* rs4880 C/T genotype and *XRCC3* rs861539 T/C (Thr241Met) genotype experienced a significant increase in grade 2 late rectal bleeding compared to patients without this particular genotypic arrangement (14% compared to 1%; $P = 0.002$). These results suggest that SNPs in the *SOD2, XRCC1* and *XRCC3* genes are associated with the development of late radiation injury in patients treated with radiation therapy for prostate adenocarcinoma.

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INTRODUCTION

With increasing implementation of prostate cancer screening programs, growing numbers of men with diagnoses of early prostate cancer face difficult decisions regarding their treatment options. Standard treatments include surgery, external-beam radiation therapy and brachytherapy. Each of these options has excellent published long-term biochemical control [i.e., freedom from prostate specific antigen (PSA) failure] and overall survival rates, although large-scale, well-conducted prospective randomized trials have not been published to allow a direct comparison of these treatments.

The limited published data leave patients and their physicians in a difficult position when deciding on treatment. Ultimately, many men reach a decision based on differences in side-effect profiles. One novel approach to assist patients faced with a diagnosis of prostate cancer may be to consider individual genetic makeup and a possible susceptibility for the development of adverse effects resulting from particular therapeutic interventions. This approach is reflective of the new era of personalized medicine in which detailed information concerning a patient's genotype will be used to decide on an optimal course of treatment that is suited specifically to that patient (*1*).

A great deal of work has been performed in recent years in an effort to identify candidate genes and single nucleotide polymorphisms in these genes that are associated with clinical radiosensitivity in a variety of cancers (*2–18*). Andreassen *et al.* (*3*) originally reported significant correlations between the development of subcutaneous fibrosis after post-mastectomy radiotherapy in 41 women and the presence of SNPs in *SOD2* (superoxide dismutase 2), *XRCC1* (X-ray repair complementing defective in Chinese hamster cells 1) and *XRCC3*. These genes are involved in cellular antioxidant defense against reactive oxygen species created by ionizing radiation, base excision repair of radiation-induced damage, and homologous recombinational (HR) repair of radiation-induced DNA double-strand

breaks, respectively. Given that disruption of one or more of these response pathways has the potential to affect the normal tissue response to radiotherapy, the goal of the current project was to screen men who received radiotherapy for prostate cancer for several common SNPs in *SOD2, XRCC1* and *XRCC3* to determine whether an association exists between any of these SNPs and the development of late radiation toxicity.

MATERIALS AND METHODS

Patients

Peripheral blood lymphocytes were collected from a consecutive series of 135 patients seen for periodic evaluation who underwent either ^{125}I or ^{103}Pd prostate brachytherapy alone or in combination with external-beam radiation therapy for early-stage prostate cancer between July 1994 and August 2004. Patient and tumor characteristics are outlined in Table 1. Brachytherapy was administered transperineally using a transrectal ultrasound probe to direct the placement of each radioactive source within the prostate (*19*). The implant characteristics are given in Table 2. The prescription dose for all ^{125}I implants was 160 Gy corrected for TG-43 recommendations (*20*). For a full ^{103}Pd implant, the prescription dose was 124 Gy using the National Institute of Standards and Technology 1999 primary calibration standard (NIST 99). For partial palladium implants, the prescription dose was 100 Gy (NIST 99). The median external-beam radiation dose was 45 Gy. Details of these treatment regimens have been described previously (*21*). Most patients (n = 101) received ^{125}I alone, while 34 men received either partial-dose ^{103}Pd combined with external-beam radiation therapy (n = 32) or full-dose ^{103}Pd alone (n = 2). External-beam radiation fields were conformal and treated the prostate and seminal vesicles using 1.5- to 2-cm margins. Patients returned at approximately 4 weeks after the implant for detailed CT-based dosimetric analysis. Patient follow-up included digital rectal examinations and serial PSA measurements, in addition to assessment of adverse response to radiation. Hormone therapy was used in 49 patients, either for downsizing in patients with large prostates (e.g. $\geq$50 cm^3) or for patients with intermediate- or high-risk features. The duration of hormone therapy was usually 3 months before and 3 to 6 months after implantation. When hormone therapy was used, it involved a gonadotropin-releasing hormone agonist alone or combined with a non-steroidal anti-androgen.

Definition of Adverse Responses to Radiation

Clinical patient data were available from the prospectively collected departmental prostate cancer database, which contained data for the 2537 patients who underwent prostate brachytherapy at Mount Sinai between June 1990 and August 2004. A detailed history was obtained and a physical examination performed before implantation followed by a directed history and physical examination at 6-month-interval follow-up evaluations. The median follow-up in this study was 53 months (range 12–140 months). The median patient age was 66 (range 46–79) years, and most patients had early-stage disease (Table 1). Late rectal toxicities were graded using the Radiation Therapy Oncology Group (RTOG)/European Organization for Research and Treatment of Cancer (EORTC) morbidity criteria (*22*). Patients who developed RTOG/EORTC grade 2 or higher late rectal effects were classified as having an adverse response. Urinary tract morbidity was measured prospectively according to the American Urological Association International Prostate Symptom Score (IPSS) sheet that was administered before the implant and at each follow-up evaluation (*23*). The urinary quality of life score from the IPSS was used for analysis, with scores of 4 (''mostly dissatisfied''), 5 (''unhappy''), or 6 (''terrible'') for long-term urinary quality of life classified as an adverse response. Erectile function was assessed using the following scoring system: 0, complete inability to have erections; 1, able to have erections but insufficient for intercourse; 2, able to have erections, sufficient for intercourse but considered suboptimal; and 3, normal erections. For this analysis, a decrease of 2 points from the pretreatment value was considered a significant decline in erection function, and these patients were classified as having an adverse response. The validity of this scoring system has been described previously (*24*, *25*). This study was reviewed and approved by the Mount Sinai School of Medicine Institutional Review Board, which oversees the ethics of research involving human subjects and the protection of the human subjects in accord with an assurance filed with and approved by the U.S. Department of Health and Human Services. Informed consent was obtained from each participant.

TABLE 1
Patient and Clinical Tumor Characteristics

Median age in years (range)	66	(46–79)
Race		
Caucasian	106	(79)
African American	19	(14)
Hispanic	7	(5)
Other	3	(2)
Erectile function		
3 (optimal)	21	(16)
2 (suboptimal but sufficient)	28	(21)
1 (suboptimal, insufficient)	29	(21)
0 (none)	33	(24)
Unknown	24	(18)
IPSS		
Good (0–7)	70	(52)
Moderate (8–19)	50	(37)
Severe (20–35)	8	(6)
Unknown	7	(5)
Urinary QOL		
0–3	122	(90)
4–6	13	(10)
Unknown	0	(0)
Alcohol use	55	(41)
Hormone use	49	(36)
Smoker	14	(10)
Hypertension	41	(30)
Coronary artery disease	12	(9)
Diabetes mellitus	8	(6)
Clinical stage		
T1b	2	(1)
T1c	77	(57)
T2a	31	(23)
T2b	18	(13)
T2c	6	(4)
Gleason score		
2–6	110	(82)
7	18	(13)
8–10	7	(5)
Median pretreatment PSA in ng/ml (range)	6.3	(0.8–43)
<10	113	(84)
10–20	16	(12)
>20	6	(4)

Notes. IPSS = International Prostate Symptom Score; QOL = quality of life; PSA = prostate specific antigen. Values are numbers (percentages) unless otherwise noted.

Detection of SNPs

DNA isolation from peripheral blood lymphocytes was accomplished using Ficoll separation as described previously (*26*). The five SNPs

TABLE 2
Treatment Regimens and Dosimetric Parameters

Implant alone	103	(76)
Combined EBRT and implant	32	(24)
Hormone therapy	49	(36)
Implant type		
^{125}I	101	(75)
Partial ^{103}Pd	32	(24)
Full ^{103}Pd	2	(1)
^{125}I median BED (Gy_2)	206	(139–259)
^{103}Pd median BED (Gy_2)	204	(169–268)
EBRT dose (Gy)	45	(39.6–70.2)
Total activity (mCi)[a]		
^{125}I	41.7	(23.5–79.2)
Partial ^{103}Pd	150	(82.6–300)
Full ^{103}Pd	221	(109–333)
D90 prostate (Gy)		
^{125}I	193	(133–239)
Partial ^{103}Pd 1	05	(53–144)
Full ^{103}Pd	145	(135–155)
D30 urethra (Gy)		
^{125}I	238	(164–419)
Partial ^{103}Pd	123	(78.1–204)
Full ^{103}Pd	166	(148–184)
V100 rectum (cm^3)	1.06	(0.01–3.04)

Notes. EBRT = external-beam radiation therapy; BED = biological effective dose; Gy_2 = unit of BED using $\alpha/\beta = 2$ for prostate cancer; D90 prostate = dose to 90% of the prostate volume on post-implant dosimetry; D30 urethra = dose to 30% of the urethral volume on post-implant dosimetry; V100 rectum = volume of the rectum receiving at least 100% of the prescription dose on post-implant dosimetry. Values are number (percentage) or median (range) unless otherwise noted.

[a] 1 mCi = 37 MBq.

screened in the three genes under investigation—*SOD2, XRCC1* and *XRCC3*—along with genotypic frequencies in this patient population are listed in Table 3. The polymerase chain reaction (PCR) primers used in this study (Table 4) were developed using an online software program (*27*). DNA forward and reverse sequencing was accomplished using an Applied Biosystems Automated 3730 DNA Analyzer (Foster City, CA), Applied Biosystems Big Dye Terminator chemistry and Applied Biosystems AmpliTaq-FS DNA Polymerase. Identification of SNPs in this patient population was performed by comparing each PCR product DNA sequence to previously published DNA sequences available on the National Center for Biotechnology Information (NCBI) website for each amplicon from each gene of interest (available online at http://www.ncbi.nlm.nih.gov/).

Statistical Analysis

Analyses were performed using the SigmaStat version 3.1 statistical software package (Systat Software, Richmond, CA). Differences in proportions were derived using Fisher's exact *t* test and odds ratios. A *P* value of <0.05 was considered to indicate statistical significance. To compare doses between different isotopes, implant alone, and combined implant and external-beam radiotherapy, biological effective dose (BED) calculations were performed as described previously (*21*) using an α/β value of 2 Gy for prostate cancer (*28–32*). Linkage disequilibrium for *XRCC1* loci in the cohort evaluable for erectile dysfunction was analyzed using Haploview software in an attempt to identify haplotypes for further analysis (*33*).

TABLE 3
Single Nucleotide Polymorphism Genotype Frequencies in the Study Population

Gene	Exon	dbSNP ref. no.	Amino acid no.	Genotype	Number (%)
SOD2	2	rs4880	16	T/T (Val/Val)	40 (30)
				T/C (Val/Ala)	72 (53)
				C/C (Ala/Ala)	23 (17)
XRCC1	6	rs1799782	194	C/C (Arg/Arg)	128 (95)
				C/T (Arg/Trp)	7 (5)
				T/T (Trp/Trp)	0 (0)
XRCC1	9	rs25489	280	G/G (Arg/Arg)	124 (92)
				G/A (Arg/His)	11 (8)
				A/A (His/His)	0 (0)
XRCC1	10	rs25487	399	G/G (Arg/Arg)	66 (49)
				G/A (Arg/Gln)	61 (45)
				A/A (Gln/Gln)	8 (6)
XRCC3	8	rs861539	241	C/C (Thr/Thr)	58 (43)
				C/T (Thr/Met)	66 (49)
				T/T (Met/Met)	11 (8)

Notes. dbSNP ref no. = National Center for Biotechnology Information Single Nucleotide Polymorphism database reference number (RefSNP Cluster Report information available online at (http://www.ncbi.nlm.nih.gov/)). Val = valine; Ala = alanine; Arg = arginine; Trp = tryptophan; His = histidine; Gln = glutamine; Thr = threonine; Met = methionine.

RESULTS

Adverse Patient Outcomes

Of the 135 men treated with radiotherapy for prostate cancer in this study, six developed RTOG/EORTC grade 2 late rectal bleeding (4%) and 13 experienced late urinary morbidity (10%). A decline in erectile function was noted in 17 of the 60 evaluable patients (28%) representing men who reported adequate pretreatment erectile function and did not receive hormone therapy. The median BED values and follow-up times (53 months) for patients in the ^{125}I and ^{103}Pd implant groups were comparable (Table 2), thereby enabling analysis of the combined set of subjects.

In addition to analyzing potential relationships between the possession of SNPs and adverse outcomes after radiotherapy, other possible variables that may predict for urinary, erectile and rectal toxicity were examined by univariate analysis (Table 5). Specifically, for the 60 evaluable patients screened for sexual dysfunction, age older than 60 was significantly associated with worse post-treatment erectile function compared to age 60 or younger [(14 of 35 (40%) compared to 3 of 25 (12%), $P = 0.01$]. For the same subset of patients, median BED, diabetes mellitus, smoking, coronary artery disease, alcohol use, hypertension, race (white or non-white), and implant type were not associated with erectile dysfunction. For the entire 135-patient cohort, these variables, as well as the rectal V100 ≥ 1.3 cm^3 [V100 = volume of the rectum receiving 100% of the prescription dose (*34*)], were not associated with late grade 2 rectal bleeding. Finally, for all patients, no potential tested variable—median BED, implant type, median urethral D30

TABLE 4
Polymerase Chain Reaction Primer

Gene	dbSNP ref no.[a]	Forward primer	Reverse primer	Fragment size (bp)
SOD2	rs4880	AGCCTGCGTAGACGGT	GAACCGGTACAAATACGAAG	379
XRCC1	rs1799782	CAGCAGCCCACCTATAATAC	CTCAACCCTACTCACTCAGG	192
XRCC1	rs25489	CCAGTGGTGCTAACCTAATC	ACACAGAGAAAGCACAAGGT	381
XRCC1	rs25487	AACTGGCATCTTCACTTCTG	TCTCAGTAGTCTGCTGGCTC	280
XRCC3	rs861539	GGGTAGGAAGGTTTTCAGAC	GCTAAAAATACGAGCTCAGG	362

[a] National Center for Biotechnology Information Single Nucleotide Polymorphism database reference number.

(D30 = dose to 30% of the urethra), and prostate volume greater than or equal to 50 cm^3—was associated with late urinary morbidity.

Rectal Bleeding and SNPs

Table 6 lists the genotypic frequencies and their association with grade 2 rectal bleeding for the entire 135-patient cohort. Patients who possessed the *SOD2* rs4880 C/T genotype exhibited a significant increase in grade 2 rectal bleeding compared to patients who had either the C/C or T/T genotype at this position (8% compared to 0%; $P = 0.02$). In addition, patients possessing the combination of the *SOD2* rs4880 T/C and the *XRCC3* rs861539 C/T genotype displayed a significant increase in grade 2 late rectal bleeding compared to patients without this particular genotypic rearrangement (14% compared to 1%; $P = 0.002$) (Fig. 1).

Urinary Morbidity and SNPs

No specific genotype or genotypic combination was significantly associated with urinary morbidity. Table 7 lists the SNPs genotyped and their association with frequency of late urinary morbidity for all 135 patients.

Erectile Dysfunction and SNPs

Table 8 lists the frequency of each genotype with respect to decline in erectile function for the 60 evaluable patients. Patients with the *XRCC1* rs25489 G/A genotype developed erectile dysfunction after radiotherapy at a significantly greater frequency than patients with the G/G genotype for this SNP (67% compared to 24%, $P = 0.048$) (Fig. 2). There was no evidence of linkage disequilibrium among the XRCC1 loci in the subset evaluable for ED (Table 9). Therefore, haplotype analysis of these markers was not performed.

DISCUSSION

Concern regarding adverse effects resulting from any cancer treatment is an issue whose importance cannot be overstated. Several modern series suggest that biochemical control rates are similar for men treated with surgery or radiotherapy for early-stage prostate cancer (*35–41*). Because of the difficulty that has been associated with conducting high-quality, large-scale randomized trials comparing therapeutic modalities, patients and their physicians lack a direct comparison of patient outcomes, including ad-

TABLE 5
Variables that may Predict for Toxicity

Variable	OR for erectile dysfunction (95% CI)	*P* value	OR for rectal bleeding (95% CI)	*P* value	OR for urinary morbidity (95% CI)	*P* value
Age >60 years	4.9 (1.2–19.5)	0.01	0.9 (0.2–4.9)	0.33	1.0 (0.3–3.4)	0.25
Smoking	0.3 (0.04–2.8)	0.21	1.8 (0.2–16.5)	0.37		
Diabetes	NA	0.36	NA	0.69		
Coronary artery disease	0.6 (0.1–3.1)	0.26	2.1 (0.2–20.0)	0.35		
Alcohol use	0.6 (0.2–2.0)	0.17	3.1 (0.5–17.3)	0.14		
Hypertension	2.0 (0.6–6.7)	0.12	1.2 (0.2–6.6)	0.33		
BED ≥205 Gy_2 (median BED)	0.8 (0.3–2.6)	0.22	1.1 (0.2–5.6)	0.32	0.4 (0.1–1.5)	0.10
Implant type (^{125}I vs ^{103}Pd)	0.4 (0.05–2.8)	0.25	0.3 (0.06–1.6)	0.13	1.1 (0.3–4.4)	0.26
Race (white compared to non-white)	1.1 (0.2–4.6)	0.29	1.4 (0.2–12.4)	0.39	0.4 (0.1–1.3)	0.08
Rectal V100 ≥1.3 cm^3			2.6 (0.5–13.4)	0.17		
Prostate volume ≥50 cm^3	0.9 (0.3–3.0)	0.24				
Urethral D30 ≥123 Gy (median D30 for ^{103}Pd)					NA	0.14
Urethral D30 ≥238 Gy (median D30 for ^{125}I)					1.8 (0.5–6.9)	0.18

Note. OR = odds ratio; CI = confidence interval; NA = not applicable; BED = biological effective dose; Gy_2 = unit of BED using $\alpha/\beta = 2$ for prostate cancer; Rectal V100 = volume of the rectum receiving at least 100% of the prescription dose on post-implant dosimetry; Urethral D30 = dose to 30% of the urethral volume on post-implant dosimetry.

TABLE 6
Rectal Bleeding and SNP Genotype Frequencies

Genotype	*n/N* (%)	Comparison	OR (95% CI)	*P*
SOD2 rs4880				
TT	0/40 (0)	TT and TC	NA	0.07
TC	6/72 (8)	TT and TC+CC	NA	0.12
CC	0/23 (0)	TT and CC NA	NA	
		TC and CC	NA	0.18
		TC and TT+CC	NA	0.02
		CC and TT+TC	NA	0.20
XRCC1 rs1799782				
CC	6/128 (5)	CC and CT	NA	0.72
CT	0/7 (0)			
XRCC1 rs25489				
GG	6/124 (5)	GG and GA	NA	0.59
GA	0/11 (0)			
XRCC1 rs25487				
GG	3/66 (5)	GG and GA	0.7 (0.1–4.4)	0.34
GA	2/61 (3)	GG and GA+AA	1.0 (0.2–4.9)	0.32
AA	1/8 (13)	GG and AA	3.0 (0.3–32.9)	0.32
		GA and AA	4.2 (0.3–52.7)	0.28
		GA and GG+AA	1.7 (0.3–9.5)	0.28
		AA and GG+GA	0.3 (0.03–2.8)	0.27
XRCC3 rs861539				
CC	1/58 (2)	CC and CT	4.7 (0.5–41.2)	0.12
CT	5/66 (8)	CC and CT+TT	4.0 (0.5–34.8)	0.15
TT	0/11 (0)	CC and TT	NA	0.84
		CT and TT	NA	0.35
		CT and CC+TT	0.2 (0.02–1.6)	0.08
		TT and CC+CT	NA	0.59

Note. *n/N* = number with adverse response/number with genotype; OR = odds ratio; CI = confidence interval; NA = not applicable.

verse effects. The available treatment options for prostate cancer are thought to be equivalent as seen in the guidelines put forth by the National Comprehensive Cancer Network (NCCN) (available online at http://www.nccn.org). Therefore, an entirely novel patient-devoted predictive test for the incidence and severity of late effects would be a welcome addition to the armamentarium of counseling physicians.

The hypothesis that guides our work, whose ultimate goal is to develop a predictive assay for the development of adverse effects resulting from radiotherapy, is that certain SNPs are associated with the development of clinical radiosensitivity. In this study, we report results suggesting an association between the *SOD2* rs4880 T/C genotype and late rectal bleeding and between the *XRCC1* rs25489 G/A genotype and a decline in erectile function after radiotherapy. In addition, possession of the *SOD2* rs4880 T/C genotype in combination with the *XRCC3* rs861539 C/T genotype was associated with a significantly increased frequency of late grade 2 rectal bleeding compared to individuals without that particular combined genotype. Finally, older age was significantly associated with worse post-treatment erectile function compared to younger age. The last finding is consistent with data reported previously (*42, 43*).

SOD2 encodes manganese superoxide dismutase, which is involved in the intracellular antioxidant defense against reactive oxygen species induced by radiation. The *SOD2* rs4880 T/C Val16Ala SNP was associated with an increased risk of subcutaneous fibrosis in a small cohort of breast cancer patients treated with post-mastectomy radiotherapy (*3*). However, a follow-up study with a larger group of women treated in similar fashion failed to show any such association (*12*). In addition to reports on radiation sensitivity, several groups have published reports associating this SNP in *SOD2* with increased susceptibility to certain cancers, including breast cancer (*44*) and B-cell lymphoma (*45*). Recently, Lan *et al.* reported no association between possession of the *SOD2* rs4880 T/C Val16Ala SNP and risk of non-Hodgkin lymphoma (*46*). Similarly, Ulder *et al.* reported no association between possession of this particular SNP in *SOD2* and prognosis in women with breast cancer (*47*).

Sutton *et al.* described the role of the *SOD2* rs4880 T/C Val16Ala SNP within the framework of the mitochondrial targeting sequence: The *Ala* variant allows for more efficient SOD2 uptake into the mitochondrial matrix and thus generates more active SOD2 compared with the *Val* variant (*48, 49*). Theoretically, a more robust response to radiotherapy may explain in part why men with the *SOD2* rs4880 T/C Val16Ala SNP appear to have increased rates of late rectal bleeding after radiotherapy for prostate cancer, as was observed in our study. Further investigation into the mechanism underlying this observation is warranted.

The cellular response to radiation-induced DNA double-strand breaks (DSBs) involves both homologous and non-homologous DNA repair pathways. *XRCC3* is involved in homologous recombinational repair of radiation-induced DSBs. Andreasson *et al.* (*12*) were unable to identify an association between increased risk of subcutaneous fibrosis and possession of the *XRCC3* rs861539 C/T Thr241Met SNP that had previously been reported in a smaller cohort of breast cancer patients treated with radiotherapy (*3*). Investigators have also reported associations between SNPs in the *XRCC3* gene and increased risk for certain cancers, including melanoma, breast, lung, AML, mesothelioma, follicular lymphoma, and bladder cancers (*50–56*). Haplotype analysis of *XRCC3* and breast cancer risk revealed an even stronger association than the individual risks associated with particular SNPs (*51*). Others have failed to show any association between SNPs in *XRCC3* and cancer risk (*44, 57, 58*).

Little is currently known about the functional significance of the *XRCC3* rs861539 C/T Thr241Met SNP. During homologous recombinational (HR) repair of DSBs, XRCC3 interacts directly with RAD51 to promote initiation of HR

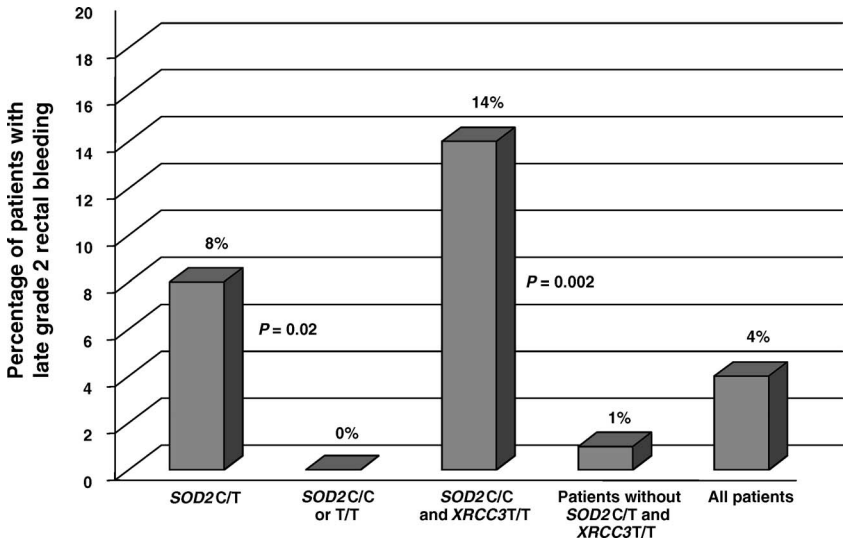

FIG. 1. Late grade 2 rectal bleeding. Patients who possessed the *SOD2* rs4880 T/C (Val16Ala) genotype exhibited a significant increase in grade 2 late rectal bleeding compared to patients who had either the C/C or T/T genotype for this SNP (6 of 72, or 8%, compared to 0 or 63, or 0%; $P = 0.02$). In addition, patients possessing the combination of the *SOD2* rs4880 T/C genotype *and XRCC3* rs861539 C/T (Thr241Met) genotype experienced a significant increase in grade 2 late rectal bleeding compared to patients without this particular genotypic arrangement (5 of 36, or 14%, compared to 1 of 99, or 1%; $P = 0.002$).

TABLE 7
Urinary Morbidity and SNP Genotype Frequencies

Genotype	*n/N* (%)	Comparison	OR (95% CI)	*P*
SOD2 rs4880				
TT	6/40 (15)	TT and TC	0.4 (0.7–8.3)	0.10
TC	5/72 (7)	TT and TC+CC	0.5 (0.7–7.1)	0.10
CC	2/23 (9)	TT and CC	0.5 (0.3–10.0)	0.25
		TC and CC	1.3 (0.1–4.3)	0.32
		TC and TT+CC	2.0 (0.2–1.7)	0.12
		CC and TT+TC	1.1 (0.2–5.5)	0.30
XRCC1 rs1799782				
CC	12/128 (9)	CC and CT	1.6 (0.2–14.5)	0.38
CT	1/7 (14)			
XRCC1 rs25489				
GG	12/124 (10)	GG and GA	0.9 (0.1–7.6)	0.40
GA	1/11 (9)			
XRCC1 rs25487				
GG	6/66 (9)	GG and GA	1.1 (0.3–3.6)	0.23
GA	6/61 (10)	GG and GA+AA	1.1 (0.4–3.6)	0.22
AA	1/8 (13)	GG and AA	1.4 (0.2–13.7)	0.40
		GA and AA	1.3 (0.1–12.5)	0.41
		GA and GG+AA	1.0 (0.3–3.0)	0.23
		AA and GG+GA	0.7 (0.1–6.4)	0.39
XRCC3 rs861539				
CC	5/58 (9)	CC and CT	1.3 (0.4–4.2)	0.22
CT	7/66 (11)	CC and CT+TT	1.2 (0.4–4.0)	0.22
TT	1/11 (9)	CC and TT	1.1 (0.1–10.1)	0.42
		CT and TT	0.8 (0.1–7.6)	0.41
		CT and CC+TT	0.8 (0.3–2.5)	0.21
		TT and CC+CT	1.1 (0.1–9.1)	0.40

Note. *n/N* = number with adverse response/number with genotype; OR = odds ratio; CI = confidence interval. NA = not applicable.

TABLE 8
Erectile Dysfunction and SNP Genotype Frequencies

Genotype	*n/N* (%)	Comparison	OR (95% CI)	*P*
SOD2 rs4880				
TT	5/18 (28)	TT and TC	1.1 (0.3–4.0)	0.25
TC	10/33 (31)	TT and TC+CC	1.0 (0.3–3.6)	0.25
CC	2/9 (22)	TT and CC	0.7 (0.1–4.9)	0.35
		TC and CC	0.7 (0.1–3.7)	0.30
		TC and TT+CC	0.8 (0.3–2.5)	0.21
		CC and TT+TC	0.7 (0.1–3.7)	0.30
XRCC1 rs1799782				
CC	16/58 (28)	CC and CT	2.6 (0.2–44.5)	0.41
CT	1/2 (50)			
XRCC1 rs25489				
GG	13/54 (24)	GG and GA	6.3 (1.03–38.5)	0.048
GA	4/6 (67)			
XRCC1 rs25487				
GG	8/24 (33)	GG and GA	0.8 (0.3–2.5)	0.21
GA	9/32 (28)	GG and GA+AA	0.7 (0.2–2.1)	0.18
AA	0/4 (0)	GG and AA	NA	0.24
		GA and AA	NA	0.30
		GA and GG+AA	1.0 (0.3–3.2)	0.23
		AA and GG+GA	NA	0.25
XRCC3 rs861539				
CC	9/26 (35)	CC and CT	0.6 (0.2–1.9)	0.16
CT	7/29 (24)	CC and CT+TT	0.6 (0.2–1.8)	0.15
TT	1/5 (20)	CC and TT	0.5 (0.1–4.9)	0.35
		CT and TT	0.8 (0.1–8.2)	0.43
		CT and CC+TT	1.5 (0.5–4.7)	0.18
		TT and CC+CT	1.6 (0.2–15.8)	0.38

Note. *n/N* = number with adverse response/number with genotype; OR = odds ratio; CI = confidence interval; NA = not applicable.

(*59*). In addition, XRCC3 appears to play a role later in HR through stabilization of heteroduplex DNA (*60*). The variant protein product of the *XRCC3* rs861539 C/T Thr241Met SNP appears to be proficient in HR repair of DSBs (*61*). In addition, transformation of human colon cancer cells deficient in *XRCC3* with the homozygous *XRCC3* rs861539 SNP gene restores HR capability (*62*). Meanwhile, Wilding *et al.* reported enhanced G_2 radiosensitivity

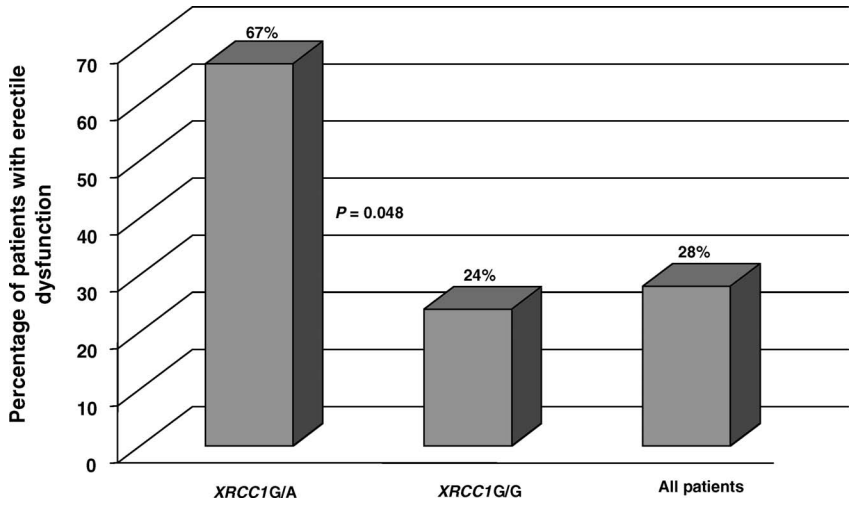

FIG. 2. Erectile dysfunction. Men with the *XRCC1* rs25489 G/A (Arg280His) genotype were more likely to develop erectile dysfunction (4 of 6, or 67%) after radiation therapy for prostate cancer than men with the G/G genotype (13 of 54, or 24%) ($P = 0.048$).

TABLE 9
Linkage Disequilibrium Analysis for *XRCC1* in Men Evaluable for Erectile Dysfunction

	rs1799782	rs25489	rs25487
rs1799782	—	1.000/0.04	0.992/0.37
rs25489	0.001	—	0.000/0.00
rs25487	0.033	0.000	—

Notes. Linkage disequilibrium analysis performed with Haploview software (*33*). D′/LOD values are above the dash marks. r^2 values are below the dash marks. None of these values are statistically significant. LOD = log of odds for disequilibrium.

in patients with the *XRCC3* rs861539 C/T Thr241Met SNP compared to controls without this polymorphism (*63*), although the mechanism behind this observation was not clear. One recent report suggested that the *XRCC3* rs861539 C/T Thr241Met SNP is associated with increased micronucleus formation in workers with occupational exposure potentially able to induce DNA strand breakage (*64*). Because a functional interaction between SNPs in *SOD2* and *XRCC3* has not been reported previously, further work is needed in this area to define the basis for our observation that this combination genotype is associated with an increased risk of late rectal bleeding.

XRCC1 coordinates base excision repair of radiation-induced DNA damage through interaction with poly(ADP-ribose) polymerase (PARP), DNA ligase III, and DNA polymerase β. SNPs in *XRCC1* have been reported to be associated with both increased (*65–67*) and decreased (*66, 68, 69*) cancer susceptibility, improved cancer prognosis (*70, 71*), and altered normal tissue response to radiotherapy (*3, 12, 13, 16*). On the other hand, several investigators have reported no association between cancer risk and SNPs in XRCC1 (*44, 54, 56, 72*). A meta-analysis by Hu *et al.* (*73*) revealed a mild reduction in overall cancer risk associated with the *XRCC1* rs1799782 C/T Arg194Trp SNP, a mild increase in overall cancer risk with the *XRCC1* rs25489 G/A Arg280His SNP, and no association with cancer risk for the *XRCC1* rs25487 G/A Arg399Gln SNP. A subsequent Japanese meta-analysis of SNPs and lung cancer risk identified an increased risk of lung cancer associated with the *XRCC1* rs25487 G/A Arg399Gln SNP among Asians but not among Caucasians (*74*). Andreasson *et al.* (*12*) failed to identify the association between increased risk of subcutaneous fibrosis and the possession of these three SNPs in *XRCC1* that had previously been reported in a smaller cohort of breast cancer patients treated with radiotherapy (*3*). Similarly, no association between clinical late toxicity after radiotherapy for treatment of prostate cancer and possession of the rs1799782 C/T Arg194Trp or rs25489 G/A Arg280His SNPs of *XRCC1* was reported by Damaraju *et al.* (*13*).

The functional significance of these three non-synonymous SNPs in *XRCC1* remains largely unknown and speculative. Musak *et al.* (*75*) recently reported observing a higher rate of chromosomal aberrations in medical workers in oncology units in Slovakia with the homozygous *XRCC1* rs25487 G/A Arg399Gln SNP than in those with the wild-type genotype. There is some evidence that SNPs in *XRCC1* may alter DNA repair capacity (*76*) or increase sensitivity of cells to ionizing radiation (*77*). In our study, the *XRCC1* rs25489 G/A Arg280His SNP was associated with a significantly increased risk of developing erectile dysfunction after radiotherapy for prostate cancer. While our results implicate an important role of the *XRCC1* rs25489 G/A Arg280His SNP in normal tissue response to radiotherapy, a better understanding of the underlying mechanism will require continued research efforts.

We were unable to find significant evidence of linkage disequilibrium among the *XRCC1* alleles in the subset evaluable for ED in our study. This may be explained by the small sample size available for analysis. As such, we were unable to perform haplotype analysis, which has more power to detect associations than studying single SNPs (*78*). In addition, race was not identified as a significant prognostic factor on univariate analysis in our study. Minor allele frequencies for these *XRCC1* loci are known to vary among ethnic groups (see http://www.hapmap.org or http://www.ncbi.nlm.nih.gov for detailed allele frequency data by ethnic group), and these differences can influence the outcome of genetic association studies. Continued work involving larger numbers of patients with various ethnic backgrounds is under way at our institution to address these important issues.

There are several limitations in this study that must be addressed. Corrections for multiple comparisons were not made, and statistical significance would not have been reached had we applied a Bonferroni correction for each SNP and effect analyzed. In addition, we did not correct for population stratification, and the relatively small number of patients experiencing late adverse effects of radiotherapy limited the statistical power from which to draw firm conclusions. From the perspective of patient safety and treatment tolerability, we would like to see these low adverse effect rates reach even lower levels in the future. At the same time, we recognize the important risk of making type I errors when statistically significantly different event rates occur in the setting of such an overall low event rate. On the other end of the statistical spectrum, it is not entirely surprising that no men with late rectal bleeding were identified in the group homozygous (genotype CC) for the *SOD2* rs4880 T/C Val16Ala SNP. We must recognize, given the limited statistical power inherent in this type of analysis, that this may represent a type II error. Therefore, the results of this study should not be regarded as definitive evidence for or against an association between these SNPs and adverse radiation response. Rather, we consider these data to be hypothesis-generating results that will require confirmation in a larger set of patients. We hope to be able to address these limitations definitively in the future as our work moves forward and our database of prostate cancer patients continues to grow.

Although the current study provides evidence indicative of an association between several SNPs in a few candidate genes with the development of radiation morbidity resulting from prostate radiotherapy, it is clear that there are likely many additional SNPs in other genes that are associated with the development of radiation morbidity. It is therefore our goal to perform a genome-wide association study to identify a greater spectrum of SNPs associated with clinical radiosensitivity. The identification of a broad range of SNPs in the human genome coupled with low-cost genotyping using high-density SNP arrays (*79, 80*) now makes this a feasible approach [see refs. (*79, 81*) and visit http://www.rtog.org for an overview of RTOG 0612 for examples of ongoing projects]. It is anticipated that through the performance of a genome-wide association study it will be possible to identify SNPs that will form the basis for a predictive assay to identify patients that are at greatest risk for the development of adverse effects resulting from radiotherapy. Using the results of such a predictive assay, radiation oncologists will be more capable of optimizing and individualizing treatment strategies. In addition, the identification of genes that possess SNPs associated with clinical radiosensitivity will provide information essential to elucidating the molecular pathways that lead to radiation injury.

In conclusion, the current study provides evidence that SNPs in the *SOD2*, *XRCC1* and *XRCC3* genes are associated with the development of late normal tissue toxicities in patients with prostate adenocarcinoma treated with radiation therapy. Future research will focus on the performance of a validation study in which a replication set of similarly treated patients will be screened for the SNPs positively identified as associated with rectal bleeding and erectile dysfunction in the current patient population. Ultimately, our goal is to conduct a genome-wide association study to identify the broad spectrum of SNPs and genes associated with radiation injury.

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CLINICAL INVESTIGATION **Normal Tissues**

ATM SEQUENCE VARIANTS AND RISK OF RADIATION-INDUCED SUBCUTANEOUS FIBROSIS AFTER POSTMASTECTOMY RADIOTHERAPY

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Purpose: To examine the hypothesis that women who are carriers of genetic alterations in the *ATM* gene are more likely to develop subcutaneous fibrosis after radiotherapy for treatment of breast cancer compared with patients who do not possess DNA sequence variations in this gene.

Methods and Materials: DNA samples isolated from fibroblast cell lines established from 41 women treated with postmastectomy radiotherapy for breast cancer were screened for genetic variants in *ATM* using denaturing high-performance liquid chromatography (DHPLC). A minimum follow-up of 2 years enabled analysis of late effects to generate dose–response curves and to estimate the dose that resulted in a 50% incidence of Grade 3 fibrosis (ED_{50}).

Results: A total of 26 genetic alterations in the expressed portions of the *ATM* gene, or within 10 bases of each exon in regions encompassing putative splice sites, were detected in 22 patients. The ED_{50} (95% confidence interval) of 60.2 (55.7–65.1) Gy calculated for patients without a sequence variation did not differ significantly from the ED_{50} of 58.4 (54.0–63.1) Gy for the group of patients with any *ATM* sequence abnormality. The ED_{50} of 53.7 (50.2–57.5) Gy for those patients who were either homozygous or heterozygous for the G→A polymorphism at nucleotide 5557, which results in substitution of asparagine for aspartic acid at position 1853 of the ATM protein, was substantially lower than the ED_{50} of 60.8 (57.0–64.8) Gy for patients not carriers of this sequence alteration. This resulted in an enhancement ratio (ratio of the ED_{50} values) of 1.13 (1.05–1.22), which was significantly greater than unity.

Conclusion: The results of this study suggest an association between the *ATM* codon 1853 Asn/Asp and Asn/Asn genotypes with the development of Grade 3 fibrosis in breast cancer patients treated with radiotherapy.

***ATM*, Breast cancer, DHPLC, Fibrosis, Radiation sensitivity.**

INTRODUCTION

Radiation-induced fibrosis (1) constitutes an important potential complication after radiotherapy (2, 3). The development of late normal-tissue reactions in breast cancer patients receiving radiotherapy shows considerable variation between individual patients. Although dosimetric variation or underlying medical conditions may be partly responsible for the morbidity, this explanation does not account for all differences between patients. Often, the adverse response is simply ascribed to unknown individual variations. However, evidence in support of genetic factors being responsible for interpatient variation in radiosensitivity is emerging, such as an examination that was performed of radiation-induced telangiectasia in breast cancer patients (4). This study described a relatively large individual variation in the progression rate to development of telangiectasia for the same radiation treatment. It was concluded that 80–90% of the variation was due to deterministic effects related to the

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existence of possible genetic differences between individuals, whereas only 10–20% of the variation could be explained through stochastic events arising from the random nature of radiation-induced cell killing and random variations in dosimetry and dose delivery.

Substantial work has been performed in recent years in an effort to identify radiosensitivity candidate genes as well as the specific single nucleotide polymorphisms (SNPs) and rare genetic variants associated with the development of adverse responses to radiotherapy (5, 6). The first gene to have received significant attention was the mutated in ataxia telangiectasia (AT) gene, *ATM*, as it was reported more than 30 years ago that patients suffering from the disease ataxia telangiectasia exhibit unusually severe and devastating responses to ionizing radiotherapy (7, 8). The ATM protein functions primarily as a protein kinase involved in cellular stress responses, cell cycle checkpoint control, and deoxyribonucleic acid (DNA) repair (9). Evidence in support for the role of *ATM* genetic variants conferring radiosensitivity to breast cancer patients comes from a study (10) in which 46 breast cancer patients were screened for *ATM* sequence variations. It was reported that 100% (3/3) of the patients that developed a Grade 3/4 subcutaneous reaction, manifested as either fibrosis or soft tissue necrosis, had *ATM* missense mutations. A second study reported a significant association specifically between homozygote carriers of the G→A transition at *ATM* nucleotide 5557 and adverse radiotherapy responses (11). In addition, evidence has been obtained demonstrating an association between *ATM* sequence variants with clinical radiosensitivity in prostate cancer patients (12, 13).

The mutation screening technique used in this study, denaturing high-performance liquid chromatography (DHPLC) (14–17), is a robust technique that can be used to screen any gene in a large population for SNPs, as well as small deletions and insertions. The advantage of DHPLC is that it enables the rapid, sensitive, and accurate identification of genetic variants in an automated fashion. Of greatest importance is the evidence that DHPLC possesses a sensitivity and specificity for DNA sequence variant detection in *ATM* approaching 100% (18).

During the period 1978–1980, postmastectomy breast cancer patients were treated in Aarhus, Denmark with a hypofractionated radiotherapy protocol. Because of a high incidence of late normal tissue complications, the fraction size was reduced to 2 Gy in 1980 (19). As a result, the majority of patients included in the present study received large doses per fraction. Skin biopsies were obtained from the patients, and fibroblasts have been cultured (20), thereby providing a source of DNA for genetic analysis. Compared with most patients treated in recent decades who have been given standard radiotherapy protocols using 1.8–2.0 Gy fraction sizes, resulting in modest normal tissue biologic doses and a relatively low incidence of late subcutaneous tissue toxicities, this Danish patient cohort represents a unique population because of the relatively large biologic doses received and the availability of skin biopsies. Furthermore, all patients in the study cohort were scored for subcutaneous fibrosis in three independent treatment fields. Differences in the dose distribution between these fields, as well as the diversity in fraction size used to treat the patients, resulted in substantial intra- as well as interpatient variation in biologically equivalent dose of 2 Gy per fraction, thereby permitting a dose–response analysis of these data. The high incidence of patients with late effects provides an ideal population to identify genetic factors associated with radiosensitivity because the doses used reached a level at which radiosensitive patients were likely to manifest a late radiation response. The relatively high biologic doses given to many patients in this cohort make this a relevant population to study in regard to treatment of tumors that require high doses to achieve control and therefore routinely result in normal tissue radiation doses in the 60–70 Gy range. In addition, the study cohort may be of particular interest considering the ongoing discussion about the ideal treatment technique (21) and fractionation regimen in postoperative radiotherapy for breast cancer (22, 23).

METHODS AND MATERIALS

Treatment characteristics, dose, and scoring of normal tissue reactions

Breast cancer patients were treated with postmastectomy radiotherapy in the Department of Oncology, Aarhus, Denmark from 1978–1982 using two fractionation protocols as previously described (19, 24). The 41 patients screened in this study represent a portion of the cohort of 319 breast cancer patients given postmastectomy radiotherapy during this period (25) and constitute the subjects for whom cultured fibroblasts were available (20). All patients were uniformly treated with a three-field technique comprising an anterior photon field, bolus area of the photon field, and an anterior electron field (Fig. 1). Thirty-four patients received 12 fractions to a minimum target dose of 36.6 Gy specified at the level of the mid-axilla or to an irradiated dose of 51.4 Gy irrespective of anteroposterior diameter. The other 7 patients were given a minimum target dose of 40.9 Gy in 22 fractions also specified at the mid-axilla. Every patient was evaluated for subcutaneous fibrosis in each individual treatment field at a single follow-up 2.2 to 5.4 years (median, 4.0 years) after completion of radiotherapy. Fibro-

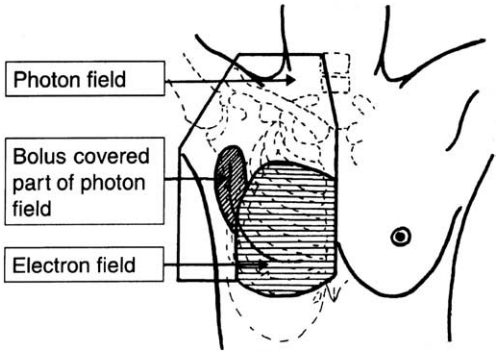

Fig. 1. Treatment field arrangement for postmastectomy radiotherapy in Aarhus 1978–1982. All patients screened in this study were treated with this technique.

sis was graded using a four-point scale identical to that later used in the Late Effects of Normal Tissue–Subjective Objective Management Analytic (LENT-SOMA) scoring system (26). Because of the large fraction sizes used for treatment of the majority of the patients, the biologic doses were often relatively high (Table 1). Therefore, Grade 3 fibrosis was detected in 37% of the individual treatment fields examined, with 56% of the patients exhibiting at least one field with this late effect.

ATM genetic screening

DNA samples were isolated from skin fibroblast cells using the Puregene DNA Isolation Kit according to the manufacturer's protocols (Gentra Systems, Minneapolis, MN). Polymerase chain reaction was used to amplify each of the 62 exons, and short intronic regions flanking each exon, that comprise the coding region of the *ATM* gene using primers previously described (18). DHPLC analysis was performed on a WAVE Nucleic Acid Frag-

Table 1. *ATM* genetic status, dose, and fibrosis in each of the 41 patients

		Photon field*		Electron field†		Bolus covered part of photon field‡	
ATM Variant	Amino acid change	Dose§	Fibrosis‖	Dose	Fibrosis	Dose	Fibrosis
5557G>A	1853D>N	43	0	52	0	56	1
5557G>A	1853D>N	52	0	62	1	69	1
5557G>A (h)¶	1853D>N	42	0	52	1	56	1
5557G>A (h)¶	1853D>N	38	0	41	0	49	0
IVS38-8T>C; 5557G>A	1853D>N	55	0	61	1	69	1
IVS38-8T>C; 5557G>A	1853D>N	42	0	41	0	50	0
735C>T; 5557G>A	245V>V; 1853D>N	57	1	61	1	69	1
378T>A	126D>E	43	0	52	0	56	0
2614C>T; 3161C>G	872P>S; 1054P>R	36	0	41	0	47	0
4258C>T	1420L>F	39	0	45	0	53	0
4258C>T	1420L>F	45	0	52	0	58	0
4258C>T	1420L>F	53	0	62	0	69	1
4578C>T	1526P>P	51	0	50	0	65	0
4578C>T	1526P>P	38	0	41	0	48	0
4578C>T	1526P>P	50	0	61	0	68	0
IVS10−6T>G	n/a	41	0	51	1	52	1
IVS62+8A>C	n/a	46	0	52	0	59	0
IVS62+8A>C	n/a	34	0	41	0	45	0
IVS62+8A>C	n/a	54	0	57	1	69	1
IVS62+8A>C	n/a	36	0	41	0	47	0
IVS62+8A>C	n/a	54	0	62	1	69	1
IVS62+8A>C	n/a	54	1	62	1	69	1
none	n/a	36	0	41	0	47	0
none	n/a	53	1	62	1	69	1
none	n/a	52	1	62	1	69	1
none	n/a	54	0	61	0	69	0
none	n/a	52	0	62	1	69	1
none	n/a	55	1	61	1	69	1
none	n/a	51	0	58	0	69	0
none	n/a	53	0	62	1	69	1
none	n/a	53	0	61	0	69	0
none	n/a	54	0	62	0	69	1
none	n/a	53	0	62	1	69	1
none	n/a	52	0	61	1	69	1
none	n/a	53	0	62	1	69	0
none	n/a	53	0	62	1	69	1
none	n/a	56	0	62	0	69	1
none	n/a	52	0	62	0	69	1
none	n/a	50	1	60	1	67	1
none	n/a	41	0	51	0	54	0
none	n/a	43	0	51	0	55	0

Abbreviation: n/a = not applicable.
* Anterior photon field including supra/infraclavicular region and axillary region.
† Anterior electron field.
‡ The part of the anterior photon field covered by a 5-mm wax bolus.
§ Equivalent dose of 2 Gy per fraction.
‖ 0 = no fibrosis, 1 = fibrosis.
¶ h = homozygote; all other variants were present in the heterozygous state.

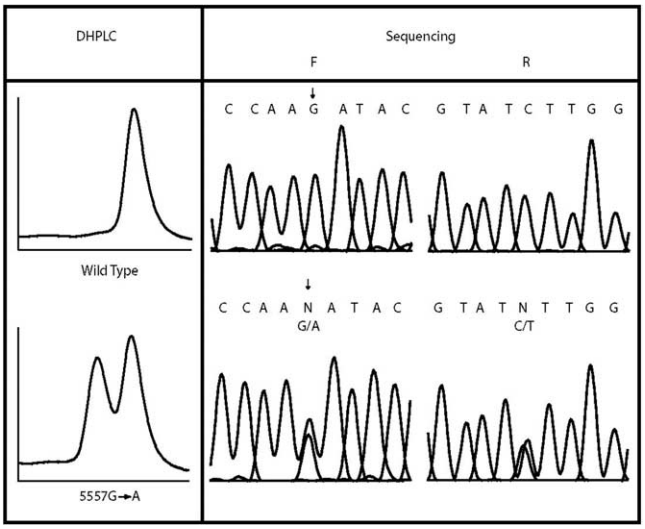

Fig. 2. Examples of wild-type pattern and genetic variant denaturing high-performance liquid chromatography (DHPLC) chromatograms. The double peak is indicative of a change in base pair sequence.

ment Analysis System (Transgenomic, Omaha, NE) using buffer gradient and temperature conditions calculated using WAVEmaker software (version 3.3, Transgenomic) designed for this purpose. An example of a wild-type and mutant chromatogram and resultant base pattern alteration is provided in Fig. 2. Exons with an aberrant DHPLC chromatogram underwent DNA forward and reverse sequencing using an ABI PRISM 377 DNA Sequencer (Foster City, CA).

Statistics and dose–response assessments

Based on exact dosimetric recordings, the physical dose absorbed at a dosimetric reference point of 4.1 mm was calculated in each field and converted into the biologically equivalent dose for 2 Gy per fraction using the linear-quadratic model (27) with an α/β ratio of 1.9 Gy for late subcutaneous fibrosis. This parameter has previously been estimated from the same dataset as used in this study (28).

Dose–response curves for patients with different *ATM* genotypes were fitted by logistic regression using the *fit model* procedure of the JMP statistical software package (SAS Institute Inc., Cary, NC). As part of this analysis, the Effect Likelihood Ratio was used to test whether the established dose–response curves differed significantly from each other. In addition, the dose that resulted in a 50% incidence of Grade 3 fibrosis (ED_{50}) was estimated by logit analysis, and differences in radiosensitivity were quantified in terms of enhancement ratios (ratios of the ED_{50} values). Ninety-five percent confidence intervals for these parameters were provided by the model (29).

The analysis was carried out for patients with any *ATM* alteration vs. those without *ATM* alterations, for patients with two alterations vs. those with less than two alterations, and for patients with and without the 5557G→A and IVS62 + 8A→C SNPs. The remaining sequence alterations could not be individually subjected to a meaningful statistical analysis as the carrier frequencies were too low to allow for dose–response assessments.

RESULTS

Table 1 provides a list of the 26 genetic alterations in the expressed portions of the *ATM* gene, or within 10 bases of each exon in putative splice site regions, that were detected in 22 of the 41 screened breast cancer patients treated with postmastectomy radiotherapy. In addition, this table lists the dose given to each field and whether Grade 3 fibrosis developed.

Figure 3 displays the dose–response for patients found to harbor any *ATM* sequence variant compared with the group of patients who did not possess an *ATM* sequence alteration. These curves did not differ significantly from each other ($p = 0.56$) The ED_{50} (95% confidence of interval) was 58.4 (54.0–63.1) Gy for the group of patients with any *ATM* sequence abnormality and 60.2 (55.7–65.1) Gy for patients without a sequence variation. This corresponded to an enhancement ratio of 1.03 (0.97–1.20). A similar analysis was performed for the patients with two *ATM* variants (6 patients, including 2 being homozygous for the 5557 G→A polymorphism), compared with those with less than two alterations. There was a trend that the dose–response curves for these groups differed from each other ($p = 0.14$) (dose–response curves not shown). The ED_{50} value for patients with two sequence alterations was 54.8 (51.3–58.5) Gy as compared with 60.5 (56.7–64.5) for those with less than two alterations. The corresponding enhancement ratio was 1.10 (1.03–1.19).

With regard to the 5557 G→A SNP, the dose–response curve for the 7 patients who were either homozygous or heterozygous for the G→A transition polymorphism was significantly different compared with the curve derived from patients without the polymorphism ($p = 0.03$) (Fig. 4). For these two groups, ED_{50} values of 53.7 (50.2–57.5) and 60.8 (57.0–64.8) Gy respectively were found, leading to an enhancement ratio of 1.13 (1.05–1.22). By contrast, no significant difference was found between the dose–response curves from the 6 patients with the IVS62 + 8A>C SNP polymor-

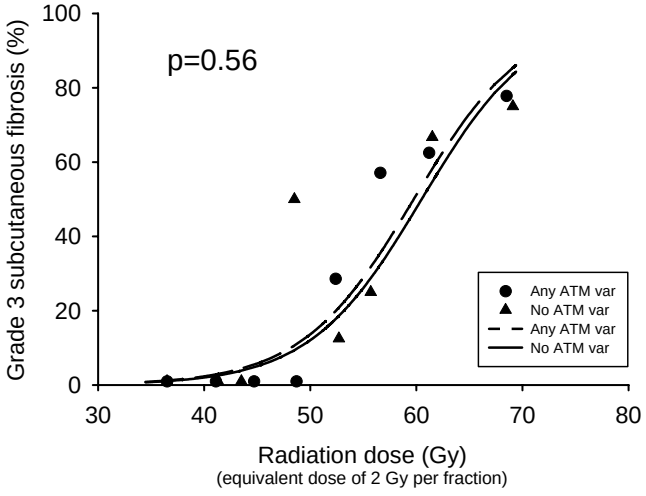

Fig. 3. Dose–response curves for subcutaneous fibrosis in patients with either any *ATM* variant or no alteration in this gene.

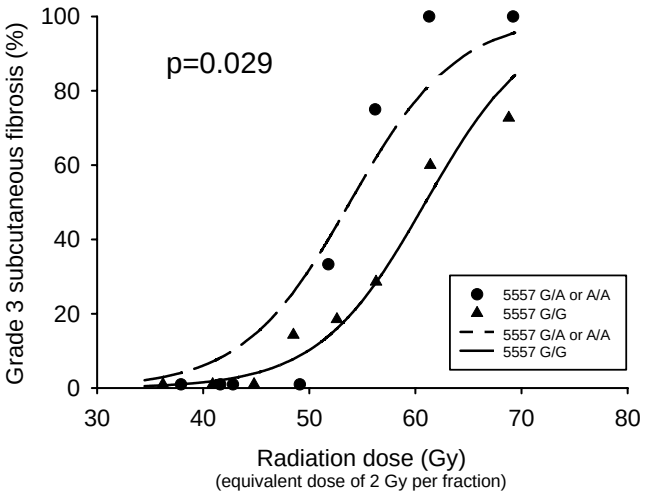

Fig. 4. Dose–response curves for subcutaneous fibrosis in patients with either the G→A polymorphism at nucleotide 5557 or not possessing this alteration.

phism and those without ($p = 0.41$) (dose–response curves not shown), or between the ED_{50} values 56.4 (50.9–62.5) and 59.9 (56.3–63.8) Gy respectively, yielding an enhancement ratio 1.06 (0.96–1.17).

DISCUSSION

Postmastectomy breast cancer patients treated with two different radiation protocols, resulting in a range of 2 Gy equivalent doses from 34–69 Gy to three fields, were screened for genetic alterations in *ATM*. Statistically significant results were obtained when the patients were analyzed with respect to the possession of the 5557 G→A SNP. Regarding the possession of two *ATM* sequence variants, a statistically significant result was found when the analysis was based on the ED_{50} estimates and enhancement ratios provided by logit analysis, whereas only a trend toward significance was found when the dose–response curves were compared by logistic regression. For these two groups, enhancement ratios of 1.13 and 1.10 respectively were found. A further analysis revealed a high degree of concordance between the group of patients with two sequence alterations and those harboring the 5557 G→A SNP (5 of 6 patients with two alterations had the 5557 G→A SNP and 5 of 7 patients with the 5557 G→A SNP had two alterations) (Table 1). Based on these observations, it seems plausible that the enhanced fibrosis risk observed among patients with two alterations was mediated by the possession of the *ATM* 5557 G→A SNP. Thus, the results suggest that women who were carriers of the 5557 G→A polymorphism developed Grade 3 subcutaneous fibrosis at lower doses compared with patients who did not possess this type of genetic alterations. In contrast, the findings of this work do not support an association between the development of fibrosis and any other *ATM* variant detected in the group of patients screened. However, we emphasize that this study provided limited statistical power to detect associations for alterations with low carrier frequencies.

Although multiple comparisons were made in this study, a Bonferroni correction (30) was not applied to the calculated *p* values, as the purpose of this study was exploratory, and it will be necessary to confirm the results of this work in a larger study. An additional issue related to the analysis of these data is that the mathematical model used to construct the dose–response curves treated the assessed radiation fields as independent data points. This approach may have resulted in an overestimation of the statistical significance as some intraindividual association may have existed between the outcomes. To address this potential problem, an analysis was performed that restricted the observations to only the bolus-covered part of the photon field (Fig. 1). This field was chosen for analysis as it had the largest range in absorbed radiation dose and provided the highest number of responses (Table 1). Even with this limitation to just one field per patient, the dose–response curves for those with or without the 5557 G→A polymorphism remained significantly different from each other when analyzed by logistic regression ($p = 0.02$) (Fig. 5). However, owing to the reduced number of observations and a smaller range in absorbed radiation dose, ED_{50} values and enhancement ratios with confidence intervals could not be determined by logit analysis.

It has previously been reported that both the incidence and severity of late normal tissue reactions after radiotherapy increase with time of follow-up (28). Although this might potentially constitute a problem, the mean follow-up time for carriers of the 5557 G>A SNP (1345 days) was nearly the same as for those patients who did not possess this variant (1399 days). Thus, the observed difference in fibrosis risk cannot be attributed to differences in length of follow-up.

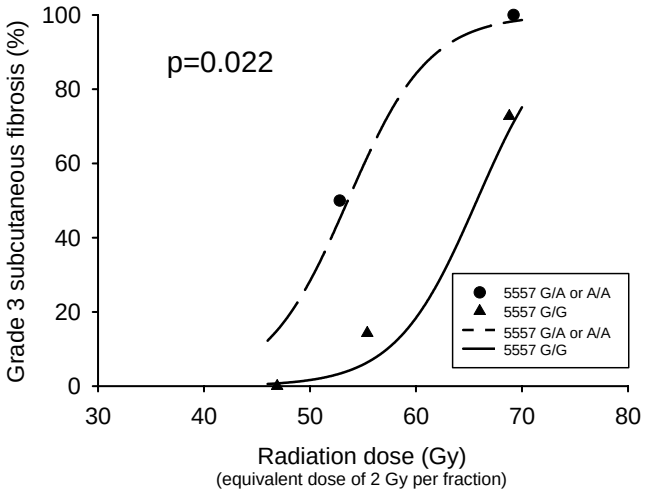

Fig. 5. Dose–response curves for subcutaneous fibrosis in patients with either the G→A polymorphism at nucleotide 5557 or not possessing this alteration when the analysis was exclusively based on observations form the bolus covered part of the photon field (i.e., one observation per patient).

Approximately 15–20% of the general population (31) possesses an adenine in place of a guanine at nucleotide position 5557 in *ATM* resulting in substitution of asparagine for aspartic acid at amino acid 1853 in the encoded protein. The results of this study are consistent with Angele *et al.* (11) who reported an association between possession of the 5557 G→A polymorphism with radiosensitivity, although the correlation found in that study was for patients homozygous for this polymorphism. In a recently published study, a nonsignificant overrepresentation of the *ATM* 5557 A allele was found among breast cancer patients with marked alterations in breast appearance after postlumpectomy radiotherapy (32). In addition, an association, which did not achieve statistical significance owing to the small sample size, was reported between this SNP and late morbidity in prostate cancer patients (12).

Although there is now substantial evidence supportive of *ATM* as a gene associated with clinical radiosensitivity, it is nevertheless highly likely that this is not the only gene whose alteration is responsible for adverse radiotherapy responses. Among the additional radiosensitivity candidate genes that have been identified as having an association with enhanced radiation responses are *TGFB1*, *XRCC1*, *XRCC3*, *SOD2*, and *hHR21*. In a previously published study based on the same patient cohort as used in the present investigation, it was observed that the risk of radiation-induced fibrosis was positively associated with the Pro/Pro genotype at codon 10 and the T/T genotype in position −509 of *TGFB1*. In addition, the *SOD2* codon 16 Val/Ala, *XRCC3* codon 241 Thr/Thr, and *XRCC1* codon 399 Arg/Arg genotypes were associated with enhanced radiosensitivity (29). Two separate studies examined polymorphic sites in *TGFB1* and also found an association between the −509 T/T and codon 10 Pro/Pro genotypes with the development of late normal tissue damage (32, 33). Another study screened three SNPs in *XRCC1* and detected an association with radiosensitivity for patients possessing either the codon 194 Arg/Trp alone or in combination with the codon 399 Arg/Gln genotype (34). It has also been reported that a T→C transition at position 1440 of the open reading frame of *hHR21* was found in 6 of 19 radiation-sensitive cancer patients (35). An important distinction between the patient population reported upon in this paper, compared with those in other studies, is that the Danish patients were not selected for screening based upon the development of late effects. Generally, it is difficult to screen unselected populations as the incidence of late effects is too low to provide a sufficient number of cases to yield statistically significant results. Because many of the patients in this study were treated with high biologic doses, there was an adequate number of subjects who developed late effects without specifically selecting patients based upon their radiation response.

As described above, associations with risk of radiation-induced fibrosis have previously been detected for SNPs in the *TGFB1*, *SOD2*, *XRCC1*, and *XRCC3* genes within the 41 patients screened in the present study. Founded on this observation, a model for estimation of fibrosis risk based on multiple SNPs was established. According to this model, the ED_{50} values for Grade 3 fibrosis correlated with the total number of "risk alleles" harbored at six polymorphic sites in these genes (29). Considering the current indications that the *ATM* 5557 G→A (codon 1883 Asp/Asn) polymorphism may also influence risk of radiation-induced fibrosis, we incorporated this SNP in a similar analysis of multiple SNPs. In the original model (29), three *TGFB1* polymorphisms (position −509, codon 10, and codon 25) were included. However, due to the existence of tight genetic linkage between these SNPs, they segregate into a limited number of well-defined haplotypes (6). Therefore, these three SNPs should probably not be regarded as independent risk factors. Furthermore, recent *in vitro* data have suggested a functional impact of the codon 10 SNP on the secretion rate of transforming growth factor beta-1 (TGFβ-1) (36). Consequently, the analysis was restricted to this *TGFB1* SNP in the current model. Thus, the Asn, Arg, Thr, Ala, and Pro alleles in *ATM* codon 1853, *XRCC1* codon 399, *XRCC3* codon 241, *SOD2* codon 16, and *TGFB1* codon 10, respectively, were defined as putative "risk alleles." The patients were grouped according to the total number of risk alleles they possessed. ED_{50} values were calculated for patients with 2–3, 4–5, and 6–7 risk alleles (Fig. 6). The patients were grouped in this way to achieve approximately the same number of subjects in each group. Because the patients segregated differently with respect to the number of risk alleles harbored, this new model could not be directly compared with the original version. However, this analysis supports the hypothesis that clinical normal tissue radiosensitivity is determined by the combined influence of multiple genetic alterations (37). Furthermore, it is noteworthy that the model identified a subset of patients characterized by a high degree of radioresistance. Nonetheless, it should be stressed that this analysis was based on a limited number of subjects and that confirmation in independent studies is needed before reaching definitive conclusions concerning a possible subpopulation of radioresistant patients.

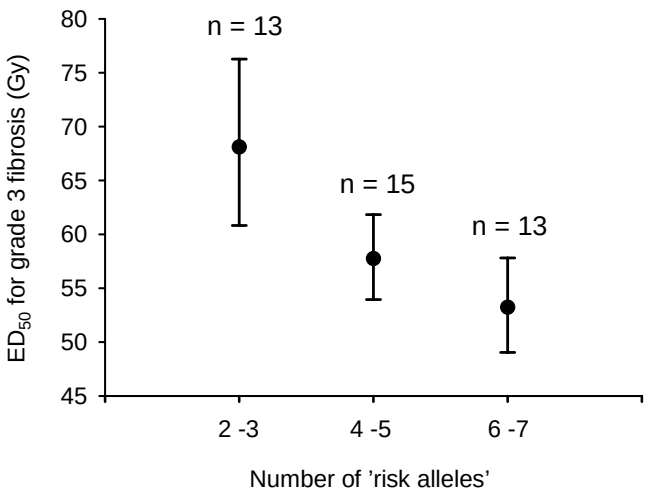

Fig. 6. Values of the dose that resulted in a 50% incidence of Grade 3 fibrosis (ED_{50}) for patients with different numbers of "risk alleles." Error bars indicate 95% confidence intervals.

CONCLUSIONS

Based upon the results of this study, a hypothesis can be formulated, which will be tested in a larger cohort of patients, that the *ATM* 5557 G>A polymorphism, resulting in the codon 1853 Asn/Asp and Asn/Asn genotypes, is associated with the development of Grade 3 subcutaneous fibrosis in breast cancer patients after postmastectomy radiation treatment.

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CLINICAL INVESTIGATION **Normal Tissues**

ATM SEQUENCE VARIANTS ARE PREDICTIVE OF ADVERSE RADIOTHERAPY RESPONSE AMONG PATIENTS TREATED FOR PROSTATE CANCER

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Purpose: To examine whether the presence of sequence variants in the *ATM* (mutated in ataxia-telangiectasia) gene is predictive for the development of radiation-induced adverse responses resulting from ^{125}I prostate brachytherapy for early-stage prostate cancer.

Materials and Methods: Thirty-seven patients with a minimum of 1-year follow-up who underwent ^{125}I prostate brachytherapy of early-stage prostate cancer were screened for DNA sequence variations in all 62 coding exons of the *ATM* gene using denaturing high-performance liquid chromatography. The clinical course and postimplant dosimetry for each genetically characterized patient were obtained from a database of 2,020 patients implanted at Mount Sinai Hospital after 1990.

Results: Twenty-one *ATM* sequence alterations located within exons, or in short intronic regions flanking each exon, were found in 16 of the 37 patients screened. For this group, 10 of 16 (63%) exhibited at least one form of adverse response. In contrast, of the 21 patients who did not harbor an *ATM* sequence variation, only 3 of 21 (14%) manifested radiation-induced adverse responses ($p = 0.005$). Nine of the patients with sequence alterations specifically possessed missense mutations, which encode for amino acid substitutions and are therefore more likely to possess functional importance. For this group, 7 of 9 (78%) exhibited at least one form of adverse response. In contrast, of the 28 patients who did not have a missense alteration, only 6 of 28 (21%) manifested any form of adverse response to the radiotherapy ($p = 0.004$). Of the patients with missense variants, 5 of 9 (56%) exhibited late rectal bleeding vs. 1 of 28 (4%) without such alterations ($p = 0.002$). Of those patients who were at risk for developing erectile dysfunction, 5 of 8 (63%) patients with missense mutations developed prospectively evaluated erectile dysfunction as opposed to 2 of 20 (10%) without these sequence alterations ($p = 0.009$).

Conclusions : Possession of sequence variants in the *ATM* gene, particularly those that encode for an amino acid substitution, is predictive for the development of adverse radiotherapy responses among patients treated with ^{125}I prostate brachytherapy.

ATM gene, Radiation sensitivity, DHPLC, Prostate cancer, Brachytherapy.

INTRODUCTION

Ataxia-telangiectasia (A-T) is a rare autosomal recessive genetic syndrome caused by genetic mutations in both copies of the *ATM* gene (1). Generally, these mutations result in truncation of the encoded protein (2). A-T is characterized clinically by cerebellar degeneration, ocular telangiectasias, and immunodeficiency. Of particular interest has been the observation that radiotherapy patients with A-T experience devastating side effects after exposure to ionizing radiation (3), including severe skin necrosis and organ dysfunction. Understanding the function of the protein encoded by *ATM* advanced greatly after cloning of the *ATM* gene. Subsequent elucidation of the activity of the ATM protein revealed a central role orchestrating the cellular response to DNA double-strand breaks (4, 5). ATM-dependent modifications of the proteins encoded by the *p53*, *BRCA1*, *CHK2*, *NBS1*, *FANCD2*, *CDC25A*, and *RAD17* genes modulate cell cycle progression and DNA repair in response to environmental assaults and ionizing radiation (6–18).

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Table 1. Patient characteristics in addition to baseline urinary, rectal, and erectile function

Characteristic	Number of patients (%)
Median age	63 years (range: 48–78 years)
Coronary artery disease	12 (32)
Angioplasty	4 (11)
Hypertension	6 (16)
Coronary bypass surgery	3 (8)
Myocardial infarction	2 (5)
Not otherwise specified	1 (3)
Active smoker	4 (11)
Reformed smoker	9 (24)
Diabetes	3 (8)
Pretreatment American Urologic Association urinary function score	
Good (0–7)	28 (76)
Moderate (8–19)	7 (19)
Severe (20–35)	2 (5)
History of transurethral prostate resection before implant	1 (3)
Preimplant ultrasound prostate volume	
≤35 cm^3	8 (22)
36–50 cm^3	20 (54)
>50	9 (24)
Erectile function	
3 - Optimal	22 (60)
2 - Suboptimal but sufficient	6 (16)
1 - Insufficient	5 (14)
0 - None	4 (11)
Ulcerative colitis/Crohn disease	1 (3)
Hemorrhoids	7 (19)

Although the occurrence of alterations in both copies of the *ATM* gene is rare, individuals who are heterozygous carriers of a single *ATM* mutation may constitute more than 1% of the general population. It has been shown that cells derived from heterozygous individuals exhibit an intermediate degree of radiosensitivity between those of wild-type and homozygously mutated cells derived from people with A-T (19–21). Animal studies have found that heterozygous $ATM^{+/-}$ mice are more susceptible to radiation-induced cataracts compared with wild-type $ATM^{+/+}$ counterparts (22). These discoveries have led to the hypothesis that possession of one altered copy of the *ATM* gene may predispose patients receiving radiotherapy to adverse reactions associated with this treatment.

Several studies have screened the *ATM* gene in patients who displayed clinically abnormal radiosensitivity. Initially, the results of these studies were negative, primarily because the samples were analyzed using a test for protein truncation (23, 24). However, it is now recognized that the most prevalent *ATM* sequence alterations detected specifically in cancer patients are missense mutations causing amino acid substitution in the encoded protein (2). In view of this understanding, further studies were conducted using assays designed to detect this class of genetic alterations, and several positive findings correlating clinical radiosensitivity and *ATM* mutations have since been reported (21, 25, 26).

Table 2. Clinical tumor characteristics

Characteristic	Number of patients (%)
PSA (ng/mL)	(range: 1.2–15, median: 6)
≤4	3 (8)
>4–10	31 (84)
>10–20	3 (8)
Gleason score	
5	5 (14)
6	31 (84)
7	1 (3)
Stage (AJCC 2002)	
T1c	25 (68)
T2a	8 (22)
T2b	4 (11)

One study, screening the *ATM* gene of 46 breast cancer patients treated with radiotherapy, revealed that 3 of 4 patients possessing an *ATM* missense mutation developed Grade 3–4 skin fibrosis. In contrast, none of the patients without a missense mutation developed this type of adverse radiotherapy response (26). Another study with a more limited genetic analysis of the *ATM* gene in which only 8 specific variants were genotyped reported that 4 of 6 breast cancer patients homozygous for the G→A transition polymorphism at nucleotide 5557, which transforms an aspartic acid into an asparagine at position 1853 of the protein, exhibited clinically abnormal radiosensitivity (25). In addition, it was reported that a patient discovered to be heterozygous for insertion of a guanine at position 3637, resulting in a frame-shift leading to a stop codon (TAG) at nucleotide 3681, experienced severe skin and subcutaneous tissue effects after conventional radiation therapy in the adjuvant setting for breast cancer (21). Cells from this patient displayed a radiosensitivity between the values for normal cells and those from patients with AT. Finally, Hall *et al.* reported that 3 of 17 prostate cancer patients exhibiting radiation-related morbidity after radiotherapy possessed *ATM* mutations (27).

The purpose of this study was to examine the hypothesis that the presence of *ATM* sequence alterations is predictive for the development of adverse radiotherapy responses among prostate cancer patients. We have screened the expressed portions of *ATM* and short adjacent intronic regions that may encompass putative splice sites for DNA sequence variations (28). This work was accomplished using denaturing high-performance liquid chromatography (DHPLC) with DNA samples derived from lymphocytes obtained from an unselected group of 37 men treated with low-dose-rate ^{125}I brachytherapy for prostate cancer. We explore any potential association of acute and late erectile, rectal, and urinary functional outcomes with *ATM* alterations using standard morbidity measuring tools.

METHODS AND MATERIALS

Patients

Peripheral blood lymphocytes were collected from a consecutive series of 37 patients seen for periodic evaluation who under-

Table 3. The postimplant dosimetric parameters of all patients

Implant characteristics	Median (range)
Total activity (mCi)	42 (27.3–62.6)
Needle number	24 (16–29)
Seed number	103 (70–171)
Dose to 90% of the prostate (Gy)	196 (156–220)
Dose to 100% of the prostate (Gy)	111 (78–139)
Volume of prostate receiving 150% of prescription dose (%)	68 (36–84.3)
Dose to 30% of the urethra (Gy)	228 (23–265)
Amount of rectum receiving 100% prescription dose (cm^3)	0.7 (0.01–3.56)

went ^{125}I prostate brachytherapy for early-stage prostate cancer between June 1997 and April 2002. All patients had biopsy-proven adenocarcinoma with central pathology review performed on all specimens. Patients were staged according to American Joint Cancer Commission standard (29). Patient and tumor characteristics are outlined in Tables 1 and 2. Brachytherapy was administered via the transperineal approach using a transrectal ultrasound probe to direct the placement of each radioactive source within the prostate (30). The implant characteristics are enumerated in Table 3. The prescription dose for all implants was 160 Gy corrected for TG-43 recommendations (31). Patients returned at approximately 4 weeks after the implant for detailed CT-based dosimetric analysis. In this study, a comprehensive dose–volume histogram analysis was available for the bladder, rectum, urethra, and prostate of each patient. Patient follow-up included digital rectal examinations and serial PSA measurements. Biochemical failure was defined using the American Society for Therapeutic Radiation and Oncology consensus definition (32).

Definition of adverse response

Patient clinical data were available from the departmental prostate cancer tissue repository database, which prospectively collected data for the 2,020 patients who underwent prostate brachytherapy at Mount Sinai between June 1990 and February 2004. All patients underwent a detailed history and physical examination before implantation followed by a directed history and physical examination at 6-month-interval follow-up evaluations. Acute and late rectal toxicities were graded using the Radiation Therapy Oncology Group (RTOG) morbidity criteria (33). Patients who developed either RTOG grade level 1 or 2 rectal effects were classified as having an adverse response. Urinary tract morbidity was prospectively measured using the American Urologic Association Symptom Score (AUASS) sheet that was administered before the implant and at each follow-up evaluation (34). The urinary quality of life score from the AUASS was used for analysis with a score of 6 or "terrible" long-term urinary quality of life classified as an adverse response. Erectile function was assessed using the following scoring system: 0, complete inability to have erections; 1, able to have erections but insufficient for intercourse; 2, can have erections sufficient for intercourse but considered suboptimal; and 3, normal erectile function. The derivation and relevance of this scoring system have been previously described (35, 36). For this analysis, a decline by 2 points was considered a significant prospective decline in erection function, and these patients were classified as having an adverse response. In addition, beginning in June 2000, the validated International Index of Erectile Function (IIEF-5) was used as a complementary method to better quantify late erectile dysfunction (ED) (37). A score of 0–2 was judged as an adverse response. The last completed form was used for this study, because the relatively recent development of the IIEF-5 did not allow for a prospective evaluation in most patients.

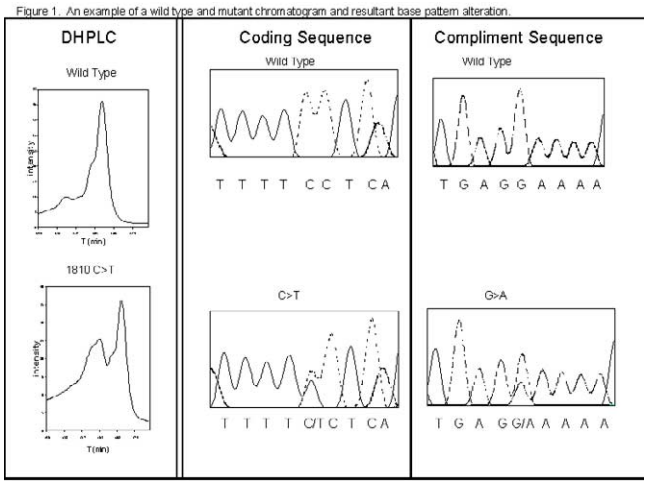

Fig. 1. An example of a wild-type and mutant chromatogram and resultant base pattern alteration.

The goals of the project were discussed with each patient as outlined by the guidelines approved in the institutional review board protocol, and written informed consent was obtained.

ATM *exon characterization*

DNA isolation from lymphocytes was accomplished using Ficoll separation as described previously (38). Polymerase chain reaction (PCR) was used to amplify each of the 62 exons, and short intronic regions flanking each exon, that comprise the coding region of the *ATM* gene using primers previously described (39). DHPLC analysis was performed on a WAVE Nucleic Acid Fragment Analysis System (Transgenomic, Omaha, NE) using buffer gradient and temperature conditions calculated using WAVEmaker software (version 3.3; Transgenomic) designed for this purpose. An example of a wild-type and mutant chromatogram and resultant base pattern alteration is seen in Fig. 1. Exons with an aberrant DHPLC chromatogram underwent DNA forward and reverse sequencing using an ABI PRISM 377 DNA Sequencer (Foster City, CA).

Statistical analysis

Analyses were performed using the Statistical Package for Social Sciences (SPSS, Chicago, IL) software. Differences in proportions were derived using the Fisher's exact *t*-test. A two-sided p value of ≤ 0.05 was considered to indicate statistical significance.

RESULTS

A total of 21 *ATM* sequence variants, representing 17 different alterations, were detected in expressed portions of the gene, or within 10 nucleotides of each exon encompassing potential splice sites, in 16 of the 37 patients screened (Table 4). It should be noted that most of the sequence variants detected in this group of patients represent genetic

Table 4. Each patient with toxicity, genetic, comorbid, and follow-up data

Patient (#)	*ATM* alteration	Prospective erectile decline	Last follow-up IIEF-5	Rectal bleeding	Urinary quality of life	D_{90} ‡ (Gy)	Comorbidities	Follow-up (months)
1	4473C>T, 149.1F>F	No	24	No	1	184	CAD	21
2		No	18	No	4	192		36
3	4578C>T, 1526P>P; 5557G>A, 1853D>N	Yes	2	RTOG 1	6	180		67
4		No	20	No	3	208	Tob	37
5		No	16	No	2	205	Tob	29
6		No	24	No	1	165		36
7		*	10	No	0	191		70
8		No	†	No	2	220		49
9	1810C>T, 604P>S	Yes	16	No	6	208		19
10	378T>A, 126D>E; IVS7-8insT; 1176C>G, 392G>G	Yes	1	No	2	197	DM	12
11	2685A>G, 895L>L; 2614C>T, 872P>S	Yes	1	RTOG 1	1	205		40
12	IVS38-8T > C	No	24	No	1	159		60
13		*	23	No	2	174	DM, CAD	31
14		No	1	No	3	210	CAD	20
15	IVS38-8T>C	No	19	No	4	164	Tob	39
16		No	14	No	0	183		59
17		*	5	No	0	169		44
18		No	22	No	2	220		40
19		No	12	No	2	206		26
20		*	21	No	2	199	Tob	37
21		*	2	No	2	174	DM, CAD	25
22	198A>C, 66K>K	*	1	No	1	217		40
23		No	23	No	1	160		25
24		Yes	9	No	2	184		39
25		*	6	No	4	218		32
26	4388T>G, 1463F>C; 1810C>T, 604P>S	*	2	RTOG 2	2	209	CAD	13
27		No	15	No	4	205		32
28	5071A>C, 1691S>R	Yes	1	RTOG 2	2	192		45
29	3161C>G, 1054P>R	No	19	No	2	197		27
30	IVS62+8A>C	No	19	RTOG 1	0	217	CAD	47
31	4578C>T, 1526P>P	Yes	8	No	0	193		26
32	2038T>C, 680F>L	No	19	RTOG 1	0	219		31
33		No	24	No	2	162		71
34		*	3	No	0	168	CAD	69
35	5557G>A, 1853D>N	No	20	No	0	186		58
36		No	18	No	1	197		43
37	IVS22-6T>G	No	22	No	3	210		29

Abbreviations: CAD = coronary artery disease; DM = diabetes mellitus; RTOG = Radiation Therapy Oncology Group; Tob = active smoker.

* Patient had a suboptimal erectile function before implant.

† Patient did not fill out IIEF-5.

‡ Dose to 90% of the prostate gland via brachytherapy.

alterations that have been previously reported as polymorphisms in *ATM* (40–42). For this group, 10 of 16 (63%) exhibited at least one form of adverse radiotherapy response. In contrast, of the 21 patients who did not harbor an *ATM* sequence variation, only 3 of 21 (14%) manifested any form of adverse response ($p = 0.005$). There were 9 patients found carrying missense mutations encoding for amino acid substitutions in the *ATM* protein. Missense mutations represent sequence alterations that are more likely to impact functional integrity. Of the 9 patients with missense mutations, 7 (78%) exhibited at least one form of adverse response. In contrast, of the 28 patients who did not have a missense mutation, only 6 of 28 (21%) manifested any form of adverse response to the radiotherapy ($p = 0.004$). Moreover, 5 of 9 (56%) patients with missense mutations exhibited an adverse response in two or three of the three organ systems evaluated (Patients 3, 9, 11, 26, and 28), whereas none of the remaining 28 patients without such sequence changes exhibited morbidity in more than one evaluated organ system ($p = 0.003$).

RTOG Grade 1 or 2 rectal bleeding was seen in 5 of 9 (56%) patients with missense mutations vs. 1 of 28 (4%) of

Table 5. Univariate analysis of variables that may predict for urinary, erectile, and rectal morbidity. All p values derived from 2-sided Fisher's exact t-test

Variable	Two radiation morbidities	SHIM erectile decline	Prospective erectile decline	Rectal Bleeding RTOG 1,2	Urinary quality of life "terrible"
Dose ≥210 Gy	1	0.34	0.29	0.14	1
Diabetes	1	0.12	0.25	1	1
Smoking	1	0.56	0.55	1	1
Coronary artery disease	1	0.17	0.55	0.32	1
ATM alteration	0.0003	0.01	0.009	0.002	0.05

Abbreviations: RTOG = Radiation Therapy Oncology Group; SHIM = Sexual Health Inventory for Men.

those without these genetic alterations ($p = 0.002$). The median amount of rectal tissue exposed to the prescription dose of 160 Gy among the individuals with rectal bleeding was 0.87 cm^3 (range, 0.04–1.24), which is below previously published rectal dosing parameters for prostate brachytherapy and predicts a low probability of late radiation-induced proctitis based upon dose alone (43).

Severe ED as quantified by IIEF-5 occurred in 5 of 9 (56%) patients with missense mutations compared with 3 of 27 (12%) of patients without these sequence abnormalities ($p = 0.01$). When considering only patients with sufficient erectile function before radiotherapy prospectively, a significant correlation was also noted between the development of erectile dysfunction in men with missense mutations, 5 of 8 (63%), as opposed to 2 of 20 (10%) in men without these types of variants ($p = 0.009$). In addition, both patients who reported a "terrible" urinary quality of life had *ATM* missense alterations (2 of 9, 22%) vs. 0 of 28 patients without missense alterations ($p = 0.05$).

The effects of total dose, diabetes, coronary artery disease, and active tobacco use were analyzed separately in relation to each of the adverse responses defined. No independent variable achieved statistical significance (Table 5), other than the presence of an *ATM* sequence alteration. In addition, none of the patients experienced a palpable local or biochemical disease recurrence.

DISCUSSION

Sixty-three percent (10 of 16) of prostate cancer patients treated with ^{125}I brachytherapy who were found to be carriers of sequence variants either within the exons or in short intronic regions flanking exons of the *ATM* gene developed at least one form of urinary, sexual, or rectal adverse response. In contrast, only 14% (3 of 21) of patients without *ATM* sequence variations displayed some form of adverse response. Furthermore, when only those patients specifically harboring missense mutations are considered, 78% of these patients developed adverse responses compared with 21% who did not possess these types of sequence abnormalities. The results of this study are supportive of the hypothesis that genetic alterations in the *ATM* gene are predictive for the development of adverse responses resulting from radiotherapy.

Radiation-induced permanent sexual dysfunction has a substantial negative impact on the quality of life of men treated for prostate cancer. Brachytherapy series have reported a widely variable incidence of reduced sexual potency after implantation (35, 36, 44–48), ranging from 14% to 50%. In this unselected series, 30% (11 of 37) of patients overall had erectile dysfunction, a figure that is consistent with previous reports. Of even greater significance, however, is that 63% of patients in this study with good preirradiation erectile function developed prospectively evaluated ED if they possessed an *ATM* missense mutation vs. 10% of men without such an alteration. The correlation of ED with *ATM* missense mutations was also apparent when men were evaluated only at last follow-up with the validated IIEF-5. Using this evaluation tool, it was found that 56% of patients with missense mutations, vs. 12% without these genetic changes, developed severe ED. These findings attest to the predictive power of *ATM* mutational status for ED and warrant validation of this striking correlation in a larger group of individuals.

A second significant correlation observed in this study is that of postradiation rectal bleeding with *ATM* sequence alterations. All of the patients who experienced late rectal bleeding had *ATM* sequence alterations. The 2 patients who manifested comparatively severe rectal bleeding, RTOG Grade 2, had DNA missense mutations. In particular, the patient with the most serious rectal bleeding was a carrier of two nonconservative missense mutations and displayed this morbidity at only 5 months after radioactive seed implantation, rather than the more typical 1.5 to 2 years. This patient underwent colonoscopy and biopsy, which identified distal proctitis and an absence of the classic telangiectasias. Patients who undergo brachytherapy receive relatively low rectal doses compared with the use of external beam irradiation involving a larger pelvic field. Most radiation-related rectal bleeding secondary to prostate cancer radiotherapy is self-limited and innocuous, but there are patients who are inordinately affected and develop rectourethral fistulas (49, 50). In these instances, it could prove even more

important to predict which patients may be radiosensitive.

With respect to the correlation of urinary symptoms with *ATM* abnormalities, the 2 patients reporting a late "terrible" urinary quality of life at last follow-up both had nonconservative missense mutations. The spectrum of affected organs for these patients included a severe decline in prospectively measured erectile function. In addition, 1 of the 2 patients had rectal bleeding. The AUASS form appears effective in quantifying the most severe urinary morbidity, but there is a relatively long symptomatic period after the implant that may decrease this instrument's power to discern differences in intermediate-term urinary function.

It may be anticipated that the tumors possessed by patients harboring ATM mutations could also be radiosensitive and that these men may exhibit higher levels of tumor control compared with patients not harboring sequence alterations. However, the patients included in this study had low-risk prostate cancer, and all were treated with optimal implants based upon evaluation of their postbrachytherapy dosimetric studies (51). It is therefore not surprising that none of the patients screened in this study failed treatment. As reported previously by our institution, these patients have an expected freedom from PSA failure of 94% at 8 years (52). Therefore, it was not possible to examine whether *ATM* genetic status conferred tumor radiosensitivity.

Clearly, there is a strong association between sequence variants in the *ATM* gene and increased clinical radiosensitivity. Nevertheless, it is highly probable that *ATM* is not the only gene whose alteration can predispose patients to adverse radiotherapy responses. Thus, the patients in this series who exhibited pronounced radiation-related morbidity, but proved negative for *ATM* sequence variants, may possess alterations in other genes associated with radiation response. Among the additional radiosensitivity candidate genes that have now been linked with enhanced radiation effects are *TGFβ1*, *XRCC1*, *XRCC3*, *SOD2*, and *hHR21* (53–56). Alterations in these genes are also likely to serve as important potential predictors of adverse radiotherapy response. In view of the clinical associations observed between radiation sensitivity and the *ATM* gene in this study, combined with the reported association of other genes, it is critical that comprehensive genetic screening of radiotherapy patients for DNA sequence variations in candidate genes associated with radiation response be accomplished, because the results of such studies could yield significant patient benefit.

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CLINICAL INVESTIGATION **Prostate**

BIOLOGICALLY EFFECTIVE DOSE VALUES FOR PROSTATE BRACHYTHERAPY: EFFECTS ON PSA FAILURE AND POSTTREATMENT BIOPSY RESULTS

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Purpose: To analyze the effect of biologically effective dose (BED) values on prostate-specific antigen (PSA) failure and posttreatment biopsy.

Methods and Materials: From 1990 to 2003, 1,377 patients had prostate brachytherapy alone (I-125 or Pd-103) (571), hormonal and brachytherapy (371), and trimodality therapy (hormonal, implant, and external beam) (435). Dose was defined as the D90 (dose delivered to 90% of the gland from the dose–volume histogram).

Results: Freedom from PSA failure (FFPF) at 10 years was 87%. The 10-year FFPF for BED <100, >100–120, >120–140, >140–160, <160–180, >180–200, and >200 were 46%, 68%, 81%, 85.5%, 90%, 90%, and 92%, respectively ($p < 0.0001$). BED and Gleason score had the greatest effect, with p values of $p < 0.0001$ in multivariate analysis. Posttreatment positive biopsy rate was 7% (31/446). The positive biopsy rates for BED ≤100, >100–120, >120–140, >140–160, >160–180, >180–200, and >200 were 24% (8/33), 15% (3/20), 6% (2/33), 6% (3/52), 7% (6/82), 1% (1/72), and 3% (4/131), respectively ($p < 0.0001$). BED was the most significant predictor of biopsy outcome in multivariate analysis ($p = 0.006$).

Conclusions: Biologically effective dose equations provide a method of comparing different isotopes and combined therapies in the brachytherapy management of prostate cancer. The effects of BED on FFPF and posttreatment biopsy demonstrate a strong dose–response relationship. © 2006 Elsevier Inc.

Prostate cancer, Brachytherapy, Biologically effective dose.

INTRODUCTION

Recent advances in the radiotherapeutic management of prostate cancer have focused on the relationship of radiation dose and tumor control. Although new technology such as three-dimensional conformal and intensity-modulated external beam radiation therapy (EBRT) has brought attention to this relationship, it is not a new area of study. Hanks *et al.*, through the patterns of care studies, helped demonstrate that increasing dose in the external beam management of prostate cancer could translate into improved local control (1). Fuks *et al.* explored the relationship between implant quality and local control in a cohort of patients treated with retropubic I-125 prostate implants and found a similar improvement in outcome with higher dose distributions (2). These early studies used the digital rectal examination to assess local control and as an endpoint for the dose–response analysis. In recent years, investigators have shifted the emphasis toward examining the relationship between dose and biochemical control. Retrospective studies have demonstrated that increasing dose over the standard 70 Gy of EBRT has resulted in improved biochemical control rates. These findings were confirmed in a randomized trial reported by Pollack *et al.* (3). In 1998, our institution established the first dose–response relationship for ultrasound-guided I-125 implants (4). Subsequent studies confirmed these findings with longer follow-up and greater numbers of patients (5, 6). In addition, brachytherapy dose–response relationships were found using posttreatment biopsy as an endpoint (6, 7). A dose–response for prostate permanent seed implantation has also been supported using other data sets (8, 9). These reports sought to explore these relationships by comparing isotope and treatment regimens. This was accomplished by looking at the implant dose derived from the postimplant dosimetric analysis as a percentage of the prescription dose. This method is problematic because prescription doses have been empirically chosen and the same percentage of a prescription dose for one isotope or treatment does not necessarily equate biologically to another.

To overcome these problems, we analyzed the dose–response relationship by developing biologically effective dose (BED) values for all brachytherapy treatments. In this

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way, different isotopes and treatment regimens (i.e., combined implant and EBRT) could be compared on a valid basis to test a dose–response relationship. In addition, the connection between local control and biochemical control was explored by testing the effect of BED on both prostate-specific antigen (PSA) failure and posttreatment biopsy results.

METHODS AND MATERIALS

A total of 1377 patients with T1 to T3 prostate cancer were treated with brachytherapy at Mount Sinai Hospital in New York from June 1990 to January 2003. No patient had radiologic or pathologic evidence of metastatic disease. All patients were staged using the 1992 American Joint Committee on Cancer staging system (10). The clinical stage, presenting Gleason score, and presenting PSA for all patients can be found in Table 1.

Seminal vesicle biopsy was performed in 609 patients (44%). Indications for performing seminal vesicle biopsy were usually high-risk features: PSA >10, Gleason score ≥7, or stage ≥T2b. Overall, 48/609 (8%) patients were found to have adenocarcinoma invading the seminal vesicles. Laparoscopic pelvic lymph node dissections were performed in 216 patients (16%), and 7 patients had pathologically positive nodes. Details of these procedures have been previously described (11).

Patients were divided into risk groups based on the presenting clinical characteristics. Low risk was defined as follows: PSA ≤10 ng/mL, Gleason score ≤6, and stage ≤T2a. Intermediate risk was defined as possessing only one of the following features: PSA >10–20 ng/mL, Gleason score = 7, stage = T2b. High risk included those with two or more intermediate risk features or one or more of the following features: PSA >20 ng/mL, Gleason score ≥8, stage T2c–T3, or positive seminal vesicle biopsy.

Treatment

All patients were treated with brachytherapy using a real time ultrasound-guided technique (12). Treatment regimens developed over time so there was overlap in different risk groups being treated by different treatment regimens. Details of the development of these treatment schemas have been previously described (13). Treatments were divided into three main groups: brachytherapy alone (571 patients), brachytherapy and hormonal therapy (371 patients), and trimodality therapy (435 patients) with brachytherapy, hormonal therapy, and external beam irradiation.

Table 1. Presenting clinical characteristics

	n	Percent
Clinical stage		
T1a	2	0.2%
T1b	9	0.6%
T1c	593	43%
T2a	292	21%
T2b	315	23%
T2c	136	9.9%
T3a	23	1.7%
T3b	3	0.2%
T3c	4	0.3%
Gleason score		
2–6	951	69%
7	282	20.5%
8–10	144	10.5%
PSA (ng/mL): range, 0.1–300, median, 7.2		
≤10	971	70%
>10–20	279	20%
>20	137	10%

Abbreviation: PSA = prostate-specific antigen.

Brachytherapy without external beam (± hormonal therapy) was performed using both I-125 (prescription dose 160 Gy, task group 43 [TG43]) (753 patients) and Pd-103 (prescription dose 124 Gy, National Institute of Standards and Technology 1999 primary calibration standard [NIST 99]) (189 patients). In general, I-125 was used for patients with Gleason scores of ≤6 and Pd-103 for those with scores ≥7. Most patients treated with brachytherapy alone were low-risk patients, although during the early years of the study period both intermediate and high-risk patients received implant alone.

Hormonal therapy and brachytherapy were employed for two main reasons. The first use of hormonal therapy was for downsizing in patients with large prostates (gland size >50 cc). It was given for 3 months before implantation and usually 2–3 months postimplant. The second use was as adjuvant therapy with brachytherapy for patients with intermediate or high-risk features. In this case, the therapy was given for 3 months before and 3 months after implantation (14).

Trimodality therapy usually involved 3 months of hormonal therapy followed by a Pd-103 brachytherapy implant (432 patients) (prescription dose 100 Gy, NIST 99) or I-125 (3 patients) (prescription dose 120 Gy) and 2 months later EBRT to a dose of 45 Gy. Seminal vesicles were implanted in patients with biopsy-positive seminal vesicle disease. The total duration of hormonal therapy was 9 months. In the earlier years of the study, lower implant doses were used with higher external beam doses. EBRT doses ranged from 39.6 to 61.2 Gy (median, 45 Gy). Details of this regimen have been previously described (15). EBRT fields were conformal and treated the prostate and seminal vesicles using 1.5- to 2-cm margins. Patients with pathologically positive pelvic nodes usually were treated with whole pelvic fields. Overall, when hormonal therapy was used it involved a luteinizing hormone–releasing hormone analog alone in 48% of patients and combined with an anti-androgen in 52%.

Dose equations

The dose delivered to the prostate was calculated using a 1-month postimplant computed tomography (CT)-based dosimetric analysis. All patients were asked to return 1 month postimplant for CT scanning. One person (R.G.S.) drew all of the prostate contours that were used to calculate prostate dose–volume histograms (DVH). Dosimetry was performed in 1321 patients. Reasons for not performing dosimetry were poor visualization due to hip prostheses or patient noncompliance. Implant dose was defined as the D90 (dose delivered to 90% of the gland from the DVH) (4). This DVH parameter was chosen because it was believed to best represent the delivered dose. This parameter has been shown to correlate well with other dose descriptions from the DVH (16). To compare doses between different isotopes and between implant alone and combined implant and EBRT, BED equations were used. The linear-quadratic model was used to determine the BED for EBRT treatments using the equation (17–20):

$$\mathrm{BED} = \mathrm{nd}\left[1 + \left(\mathrm{d}/\alpha/\beta\right)\right] \quad (1)$$

where n = number of fractions; d = dose per fraction; and α/β = a tissue and effect specific parameter associated with the linear-quadratic model. The equation used to calculate the BEDs for the low-dose-rate permanent decaying implants with I-125 and Pd-103 was (21):

$$BED = (R_0/\lambda)\{1 + [R_0/(\mu+\lambda)(\alpha/\beta)]\} \quad (2)$$

where R_0 = initial dose rate of implant = (D90)(λ); λ = radioactive decay constant = $0.693/T_{1/2}$; $T_{1/2}$ = radioactive half-life of isotope; μ = repair rate constant = $0.693/t_{1/2}$; and $t_{1/2}$ = tissue repair half-time. The specific values used for these constants for prostate carcinoma were α/β = 2 Gy, $t_{1/2}$ = 1 h, $T_{1/2}$ = 60 days for I-125 and 17 days for Pd-103 (22–25).

The BED values for treatments involving both implant and EBRT were calculated by adding the BEDs computed for each treatment (26). Because prostate cancers typically have a relatively long median potential doubling time of approximately 42 days and it is uncertain as to when accelerated repopulation begins after treatment is initiated, a correction for tumor cell repopulation was not included in these calculations (27, 28). However, this modification to the BED may be of significance for those prostate cancers that exhibit more aggressive cell repopulation during an implant treatment (29).

Follow-up

All patients were asked to return every 6 months after completion of treatment. Follow-up consisted of calling referring physicians as well as sending out mailed questionnaires. Follow-up was calculated from completion of treatment to last available follow-up date or date of death and ranged for the entire population from 2 to 14 years (median, 4.2 years). PSA failure was determined using the American Society for Therapeutic Radiology and Oncology definition (30).

Overall, 446 patients underwent posttreatment biopsies. Biopsies (8–10 core samples) were recommended at 2 years posttreatment. Repeat biopsies after this point were done for the following reasons: initial negative biopsy with continued rise in PSA or initial positive biopsy with no evidence of a rising PSA. The outcomes for the biopsies were based on the last biopsy performed. Overall, 370 patients underwent 1 biopsy, 53 underwent 2 biopsies, 17 underwent 3 biopsies, 5 underwent 4 biopsies, and 1 underwent 5 biopsies. Biopsy results were read as positive or negative with no indeterminate group (7). In general, patients with rising PSA profiles were more likely to consent to biopsy than those with stable PSA levels. Eighteen percent of those patients receiving posttreatment biopsies experienced a PSA failure vs. 5% of those who did not undergo biopsy ($p < 0.0001$). Those receiving a biopsy were followed from 2 to 13.8 years (median, 6.5 years).

BED groupings

For the purpose of this analysis, patients were divided into seven BED dose groups. The groups were ≤100 (45 patients), >100–120 (32 patients), >120–140 (51 patients), >140–160 (97 patients), >160–180 (191 patients), >180–200 (311 patients) and >200 (594 patients). In general, patients treated during the early years of the study period received lower BED values. The median follow-up in years for the seven groups were as follows: 9.2, 8.3, 7.2, 6.8, 5.6, 3.7, and 3.6, respectively. Although the median follow-up was longer for patients with lower BEDs, the number of patients in each group increased sharply in the higher BED groups. The number of patients at risk for the actuarial analyses were similar for high and low BED groups. When patients were divided into two BED groups, <150 (169 patients) and ≥150 (1152 patients), their median follow-up times were 7.7 and 3.9 years, respectively. The number of patients at risk greater than 7.7 years were similar for the two groups with 83 patients in dose group <150 vs. 81 patients in dose group ≥150.

Statistics

Survival curves were determined using the methods of Kaplan and Meier. Differences in survival rates were calculated using the log–rank test. Multivariate analysis of survival was performed using a Cox-regression analysis. Differences in proportions were tested using the chi-square test. Logistic regression was used to test the effect of multiple variables on biopsy outcomes (31).

RESULTS

Biochemical control

The overall freedom from PSA failure (FFPF) for the whole cohort at 10 years was 87% (Fig. 1). Patient age had a significant effect on PSA failure. Ten-year FFPF rates for patients <60 (305), >60–70 (659), and >70 (413) were 91%, 87%, and 85%, respectively ($p = 0.03$). The FFPF rates broken out by presenting disease characteristics can be found in Table 2. All of the disease characteristics significantly affected FFPF in univariate analysis. The choice of treatment did not significantly affect PSA failure, with FFPF rates at 10 years of 85%, 91%, and 88% for treatment groups implant alone, implant plus hormonal therapy, and trimodality therapy, respectively ($p = 0.22$). In addition, the use of hormonal therapy also did not significantly affect 10-year FFPF, with rates of 85% for those not receiving hormonal therapy vs. 89% for those treated with hormonal therapy ($p = 0.33$).

The effect of BED groups on FFPF was analyzed, and results can be seen in Fig. 2. There was a significant improvement in FFPF rates with increasing BED doses. The

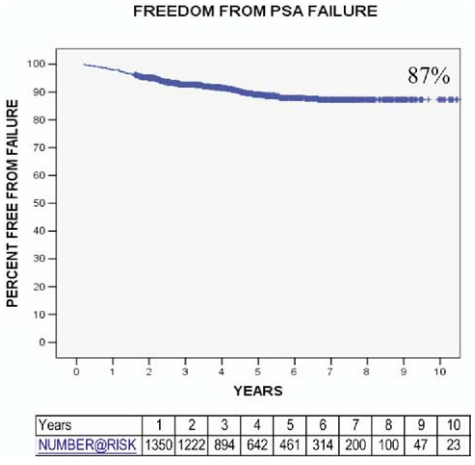

Fig. 1. Freedom from biochemical failure for entire study population. PSA = prostate-specific antigen.

Table 2. Effect of disease-related factors on PSA failure

Factor	10-year FFPF	*p* Value
PSA		
≤10	90%	
>10–20	85%	
>20	70%	<0.0001
Gleason score		
≤6	90%	
7	85%	
≥8	76%	<0.0001
Clinical stage		
<T2a	93%	
≥T2b	78%	<0.0001
Risk group		
Low	94%	
Intermediate	89.5%	
High	78%	<0.0001

Abbreviations: FFPF = freedom from PSA failure; PSA = prostate-specific antigen.

10-year FFPF for the BED groups <100, >100–120, >120–140, >140–160, >160–180, >180–200, and >200 were 46%, 68%, 81%, 85.5%, 90%, 90%, and 92%, respectively ($p < 0.0001$). Dichotomizing the data based on the above findings into two BED dose groups <150 (169 patients) and ≥150 (1152 patients) revealed FFPF rates at 10 years of 69% and 91%, respectively ($p < 0.0001$) (Fig. 3). The distributions of treatment regimens among the two dose groups were as follows: dose group <150 (52% implant alone, 38% implant and hormonal therapy, 10% trimodality), dose group ≥150 (40% implant alone, 24% implant and hormonal therapy, 36% trimodality). A multivariate analysis was performed using Cox regression with the enter model and variables entered at a level of 0.05 and removed at a level of 0.1. Disease and treatment variables were analyzed as categorical data as stratified in the univariate analyses. *p* values for the variables can be found in Table 3.

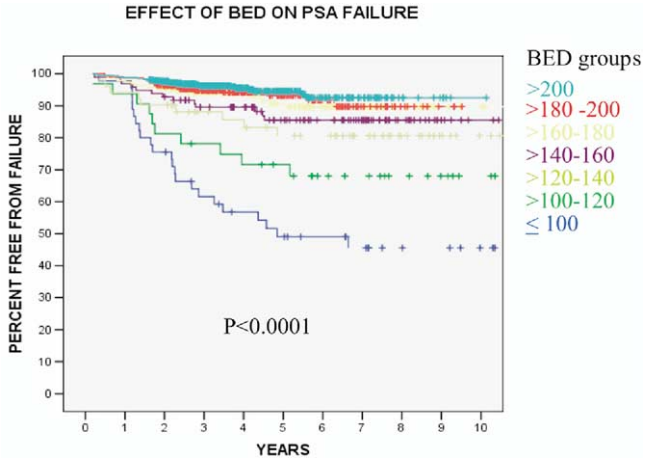

Fig. 2. Effect of biologically effective dose (BED) groups on biochemical failure. PSA = prostate-specific antigen.

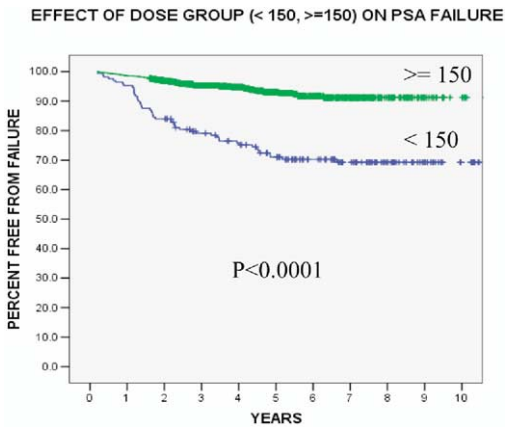

Fig. 3. Effect of biologically effective dose cutpoint of 150 on biochemical failure. PSA = prostate-specific antigen.

In this analysis, Gleason score and BED had the greatest impact on PSA failure. In addition, a multivariate analysis was performed using BED as a continuous variable. This demonstrated similar results with *p* values for BED, Gleason score, PSA, treatment, hormonal therapy, risk group, stage, and age as follows: 0.000, 0.000, 0.013, 0.199, 0.222, 0.49, 0.09, and 0.43, respectively.

Patients with high-risk features are the ones most likely to harbor microscopic disseminated disease at presentation. In theory, if a significant portion of high-risk patients have subclinical metastatic disease, then local control of disease would have little effect on biochemical failure rates. In this scenario, it would be difficult to demonstrate a dose–response analysis. To explore this hypothesis, dose–response analyses were performed on subsets of high-risk patients. Because these subgroups contained smaller numbers of patients compared with the whole population, dose–response analyses were limited to separating patients into two BED groups, <150 and ≥150. Using this type of analysis, BED significantly affected freedom from biochemical failure rates in patients with Gleason scores 8–10,

Table 3. Multivariate analysis of factors affecting PSA failure

Factor	*p* Value	Exp(B)	95% confidence interval for Exp(B) Lower	Upper
Age	0.303	1.16	0.877	1.52
Treatment group	0.225	0.749	0.469	1.19
Clinical stage	0.103	1.59	0.911	2.79
Risk group	0.326	1.27	0.79	2.03
Hormonal therapy	0.178	0.637	0.331	1.23
PSA	0.012	1.45	1.08	1.93
Gleason score	0.000	1.72	1.29	2.31
BED	0.000	0.741	0.652	0.843

Abbreviations: BED = biologically effective dose; PSA = prostate-specific antigen.

Table 4. BED dose response analyses in high-risk subgroups

BED	*n*	10-year FFPF	*p* Value
Gleason score 8–10			
<150	28	60%	
≥150	110	82%	0.003
Clinical stage >T2b			
<150	96	60%	
≥150	355	86%	0.0001
PSA >20			
<150	30	45%	
≥150	83	79%	<0.0001
High risk			
<150	97	61%	
≥150	343	83%	<0.0001

Abbreviations: BED = biologically effective dose; FFPF = freedom from PSA failure; PSA = prostate-specific antigen.

patients with stage >T2b, patients with PSA >20, and in the subgroup of high-risk patients (Table 4).

Biopsy results

The overall last positive biopsy rate was 7% (31/446). The effect of disease and treatment factors on biopsy outcomes can be found in Table 5. There was a significant association of lower positive biopsy results with increasing BED dose groups (Table 6). The division of patients into two BED dose groups (<150, ≥150) revealed positive biopsy rates of 13% (14/110) and 4% (13/312), respectively ($p < 0.0001$). A logistic regression analysis of factors potentially affecting last biopsy results, using categorical variables as described above, was performed; results are presented in Table 7. This revealed that BED was the most significant predictor of last biopsy results. In addition, the effect of BED on biopsy outcome was tested in the high-risk patient subgroup. In these patients, BED continued to show a significant effect. Patients receiving a BED <150 had a positive biopsy rate of 15% (9/61) compared with 3.5% (3/85) for patients with BED ≥150 ($p = 0.015$).

Table 5. Effect of disease and treatment factors on last biopsy results

Factor	Positive biopsy rate	*p* Value
PSA		
≤10	6% (17/300)	
>10–20	8% (8/104)	
>20	14% (6/42)	0.11
Gleason score		
≤6	7% (22/330)	
7	9% (6/70)	
8–10	6% (3/46)	0.84
Clinical stage		
≤T2a	4% (11/270)	
>T2b	11% (20/176)	0.003
Risk group		
Low	4.5% (8/176)	
Intermediate	6% (7/110)	
High	10% (16/160)	0.14
Treatment		
Implant alone	10% (24/232)	
Implant + HRM Rx	3% (4/145)	
Trimodality	4% (3/69)	0.01
Hormonal therapy		
Yes	3% (7/207)	
No	10% (24/239)	0.006

Abbreviations: HRM Rx = hormonal therapy; PSA = prostate-specific antigen.

Table 6. Effect of BED values on posttreatment biopsy results

BED groups	Number of patients	Percent positive	
≤100	33	24%	
>100–120	20	15%	
>120–140	33	6%	
>140–160	52	6%	
>160–180	82	7%	
>180–200	72	1%	
>200	131	3%	$p < 0.0001$

Abbreviation: BED = biologically effective dose.

Relationship between biopsy results and biochemical failure

Patients experiencing a PSA failure were more likely to have their last biopsy read as positive (22% [18/81]) compared with those without PSA failure (4% [13/365]) ($p < 0.001$). Conversely, patients with a positive last biopsy had a decreased FFPF rate at 10 years of 36.5% compared with 83% for those patients with a negative last biopsy ($p < 0.0001$).

DISCUSSION

In 1998, we published our first report on the effect of implant dose on biochemical outcome using I-125 implants (4). The decision made at that time was to perform the dose–response analysis only on one isotope to avoid having to compare doses between two isotopes with different half-lives and dose rates. In addition, patients treated with combined implant and EBRT were also excluded for similar reasons. In 2000, we analyzed the effect of dose on posttreatment biopsy outcomes. Be-

Table 7. Logistic regression analysis of factors affecting last biopsy results

Factor	*p* Value
PSA	0.88
Gleason score	0.83
Risk group	0.67
Treatment group	0.34
Hormonal Rx	0.05
BED	0.006

Abbreviations: BED = biologically effective dose; PSA = prostate-specific antigen; Rx = therapy.

cause of our concerns of differences in dose rate, total prescription dose, and half-lives, analyses were performed separately for I-125 and Pd-103 implants. Both analyses revealed significant effects of dose on biopsy outcomes (7). Based on these findings, we created an optimal dose group and suboptimal dose group that would enable both isotopes to be analyzed together. The optimal dose group was ≥140 Gy for I-125 implants and ≥100 Gy for Pd-103 implants, and the suboptimal group was considered those with lower doses. Using this grouping, we subsequently reported, in an updated report, a dose–response relationship (5). The problem with this type of analysis was that treatments were lumped into only two groups and more subtle differences in doses could not be tested. In addition, this analysis continued to exclude patients treated with combined implant and EBRT. To rectify these problems, we decided to derive BED values for all patients receiving prostate brachytherapy.

One of the crucial elements of the BED formulas is the α/β ratio. It is generally accepted that most tumors display modest sparing because of fractionation and therefore are characterized by relatively high α/β values in the range of 10 Gy (32). In contrast, accumulating evidence has been obtained consistent with the conclusion that prostate cancers display a substantial sensitivity to fractionation and thus possess a relatively low α/β ratio in the range of 1–3 Gy (22, 24, 25, 33, 34). In fact, one of the early articles to recommend a low α/β, authored by Brenner *et al.*, used our initial 1998 I-125 dose–response data to postulate that the α/β for prostate cancer should be 1.5 (4, 22). We therefore selected a value of 2 Gy for the calculations presented in this study.

Using these BED values, we were able to demonstrate a significant dose–response relationship between increasing BED and higher biochemical control and negative biopsy rates. Ten-year FFPF rates increased from 46% to 92% as BED values increased from <100 to >200 using BED increments of 20. Using these same BED groupings, last posttreatment positive biopsy rates dropped from 24% to 3%. The lower BED patients tended to be treated in the early years of the study period, when our implant technology and experience was in its infancy. For this reason, patients in the lower BED groups had longer follow-up with the group <100 having a median follow-up time of 9.2 years compared with 3.6 years for BED group >200. Although this observed difference could never really be corrected, the greater number of patients treated per year over time allowed for fair comparisons among dose groups. In dichotomizing the data, the number of patients at risk in the actuarial analysis past 7.5 years is actually the same in the low-dose and high-dose groups.

Although this is the first study to use BED equations to demonstrate a dose–response relationship, other investigators have also shown a relationship between implant dose parameters and biochemical control rates. Potters *et al.* found that for I-125, Pd-103, and combination therapy treatments, patients whose D90 values were <90% of the prescription dose had a 5-year FFPF of 82% compared with 93% for those with D90 values ≥90% of the prescription dose (8). Wallner *et al.* demonstrated in a randomized trial of Pd-103 vs. I-125 implants, that those patients receiving a D90 > 100% of the prescription dose had a 98% FFPF vs. 82% for those with D90 < 100% (9).

The present study also explores the relationship between local control as measured by posttreatment biopsy outcomes and biochemical failure. It appears that local control is the driving force behind biochemical control. Patients with a positive last biopsy had a decreased FFPF rate at 10 years of 36.5% compared with 83% for those patients with a negative last biopsy. The analysis of the effects of BED on PSA failure and biopsy results further supports this theory. Increasing dose to the prostate primarily improves outcomes by enhancing local control. This is supported by the fact that BED was the most significant predictor in multivariate analysis of both biochemical control and posttreatment biopsies. The significant effect of BED on FFPF rates in high-risk patients supports the theory that higher risk patients probably have greater tumor burdens and need higher doses for control. This indirectly supports the use of combined implant and EBRT (the treatment regimen with the highest associated BED values) for high-risk patients. Whether these high BED values can be achieved by delivering higher than currently prescribed doses for brachytherapy alone is a question that can not be addressed within the scope of the current report.

In addition, this BED effect contradicts the theory that most high-risk patients who develop PSA failure after local therapy do so because of the presence of microscopic disseminated disease, a theory commonly used to explain the poor results of radical prostatectomy in high-risk patients. The dose–response seen in high-risk patients demonstrates that local control plays an important role in controlling disease progression in this subset of patients.

These results continue to support the routine use of postimplant dosimetry to analyze implant quality. Implants or treatments that yield BED values less than 150 should be examined on a case by case basis. Low BED implants can be potentially addressed with reimplantation or the addition of supplemental external beam irradiation (35). Hopefully, these BED findings can be used as a guide in this setting.

In conclusion, BED equations provide a method of comparing different isotopes and combined therapies in the brachytherapy management of prostate cancer. The effects of BED on FFPF rates and posttreatment biopsy outcomes continue to demonstrate a strong dose–response relationship. The results of the current analysis support the recommended brachytherapy dose prescriptions but emphasize the need for the treatments to achieve their stated dose goals. If current brachytherapy prescription doses can be achieved, excellent long-term biochemical and local control rates can be realized.

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Brachytherapy for the Treatment of Prostate Cancer

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Abstract: Low-dose rate brachytherapy has become a mainstream treatment option for men diagnosed with prostate cancer because of excellent long-term treatment outcomes in low- , intermediate- , and high-risk patients. Largely due to patient lead advocacy for minimally invasive treatment options, high-quality prostate implants have become widely available in the US, Europe, and Japan. The reason that brachytherapy results are reproducible in several different practice settings is because numerous implant quality factors have been defined over the last 20 years, which can be applied objectively to judge the success of the intervention both during and after the procedure. In addition, recent long-term follow-up studies have clarified that the secondary cancer incidence of brachytherapy is not clinically meaningful. In terms of future directions, the study of radiation repair genetics may allow for the counseling physician to better estimate any given patients risk for side effects, thereby substantially reducing the therapeutic uncertainties faced by patients choosing a prostate cancer intervention.

Key Words: prostate cancer, prostate brachytherapy, minimally invasive techniques

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The use of low dose rate brachytherapy using computer-assisted treatment planning confers a reproducible cure rate with a limited side effect profile.[1] The first conception of the transperineal approach using a transrectal ultrasound probe was initially reported by Holm in 1983. Over the last 25 years numerous significant advances have been made, which built on this initial insight.[2–4] In this review we will first describe the various methods that have evolved relating to a transperineal approach. After this discussion, we will review the efficacy and side effects of prostate brachytherapy.

Outcomes derived from a broad range of brachytherapy experience, including results from large institutions with 20 years of experience, as well as results from groups reporting their initial cases, will be presented. This diversity of excellent results is arguably the main strength of prostate brachytherapy relative to the other available methods for the treatment of localized prostate cancer. Low dose rate brachytherapy pioneers carefully delineated implant quality factors, which has provided a solid foundation upon which reproducible results are possible across a spectrum of clinical experience.[5–7]

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IMPLANT TECHNIQUES

Real Time

In 1990 physicians at the Mount Sinai Medical Center developed the prostate brachytherapy technique termed the real-time method. This technique is heavily reliant on detailed clinical knowledge of the transverse and sagittal ultrasound anatomy of the prostate gland. According to the original inception of this method, an activity per volume table (nomogram) is used to find the proper amount of activity for the seeds to be implanted. Based on the concepts put forth by Patterson and Parker, a peripherally weighted implant can be completed by following a relatively straightforward set of guidelines.[4]

The first step, usually performed in the urologist's office, is determination of prostate volume by applying an ellipsoid formula (height × width × length × 0.52). This volume is used to determine the number of seeds and total activity ordered for the patient by referring to a look-up table. In the operating room, the prostate volume is remeasured using step-section planimetry at 5-mm intervals from base to apex. Three longitudinal measurements (anterior, middle, and posterior) of the prostate are made in the midline to find the average length of the gland; this important step serves as a general guide for the number of seeds to be placed within the periphery and interior of the gland. The suggested seed activities for both I-125 (range 0.3–0.6 mCi) and Pd-103 (1.5–3 U) are titrated as such to give a continuous isodose line with each other if placed no further than 1cm apart. Therefore, a prostate length of 3 cm will require 4 seeds, 1 at both the apex and base and 2 in the middle. A 4-cm length will require 5 seeds and so forth. The number of peripheral needles is determined by taking a circumferential measurement at the prostate's greatest transverse diameter. If the circumference is 12 cm, then at least 12 needles should be used. The final decision on the number of needles and spacing between needles and seeds will be somewhat dependent on the activity per seed selected. A higher activity will allow greater spacing (and therefore fewer needles and seeds) but at a cost of needing to be more conservative with proximity to the urethra and rectum. These simple measurements, in addition to referencing the look-up table, allow one to have a reliable road map for the seed implant without the use of a computer-mediated plan. In addition, it allows the implant

team to work from the same set of reproducible assumptions to evaluate new technologies or software innovations.[8,9]

The implantation is intentionally divided into an initial peripheral and subsequent interior phase. Placing the peripheral needles only greatly improves the imaging of the anterior needles and seeds. As this technique is highly dependent on direct visualization of the position of each needle and seed, any interference caused by the interior needles could contribute to an inferior dosimetric outcome. The goal of the interior needle and seed placement is to deliver the dose to the base and apex of the gland, to provide dose escalation if desired, and to supplement any "cold" areas not covered adequately by peripheral seed placement.

The initial phase consists of peripheral needle placement (usually 12–18 needles) just inside the prostate capsule, with approximately 1-cm spacing, using the greatest transverse image of the prostate. During this process, it is important, although not imperative, that bilateral needle symmetry is achieved within the prostate. Although the length of the prostate gland and the number of needles required are known, the precise number of seeds required may need to be adjusted on the basis of the evaluation of the intraoperative treatment plan, which is developed in tandem with placement of the peripheral needles. When centers are first starting utilization of this technique, an even loading of the peripheral needles from base to apex as identified on the sagittal image, which depicts the entire length of the Foley catheter, would be recommended. For centers with experience, integration of the treatment planning computer into the determination of precise needle and seed placement can allow for extremely conformal implants that simultaneously deliver an oncologically optimal dose to the entire gland while avoiding hot spots near the urethra. From a technical standpoint, it is important to also remember that the posterior needles should be placed well beyond the prostate capsule into the prostate parenchyma, at least 5 mm from the capsule and/or 8 mm from the inner rectal mucosa. This will assure that the rectal dose is well within the acceptable range. Generally, 75% of the seeds are placed in the periphery, in accordance with the principles of Paterson and Parker. If one uses an intraoperative treatment planning software system, it is very common, especially in smaller glands $<35 cm^3$ for 80%–85% of the seeds to be deposited in the periphery of the gland. After insertion of peripheral needles to take into account the effect of edema, prostate deformation, and precise needle position, the images are again recaptured in the treatment planning system and the initial plan is reevaluated in light of the new position of the prostate. This reevaluation serves as an additional opportunity to optimize the implant dosimetrically and to critically evaluate peripheral needle distribution. The radioactive sources are placed individually using the sagittal setting of the probe. It is important for the brachytherapist to identify prostatic anatomy before the placement of the sources by referring often to the midline sagittal image to ensure that probe movement and prostate movement are properly accounted for as the sources are placed throughout the peripheral needles. In addition, during this process the exact seed position is mapped by the dosimetrist using the real-time treatment planning software. If a seed slips or clumps, this event is accounted for and its consequences can be evaluated and adjusted for during the remainder of the implant procedure.[10] For source placement, the Mick applicator (Mick TP-200; Mick Radionuclear Instruments, Mount Vernon, NY) is used. It is important to remember that the prostate is a three-dimensional object in terms of its relations to the bladder, urethra, and rectum. The Mick applicator allows the operator the freedom to place seeds closer together or farther apart from each other as required by an individual's anatomy. This is particularly important for insertion of the peripheral apical seeds. Here the prostate anatomy is best visualized by sagittal imaging, and a mechanistic approach is best avoided to ensure that seeds are not placed into the periprostatic tissue, which at this point consists largely of perirectal musculature. In addition, the treatment planning system allows the brachytherapist to judge in 3 dimensions where he or she is at in relation to the urethra throughout the procedure. For order of needle implantation, it is best to be consistent in approach to allow the dosimetry team to follow the progress of the implant accurately. Generally the needles furthest from the probe are the most difficult to visualize and should be implanted first, with progression toward the posterior needles near the probe.[11]

After the entire periphery is implanted, insertion of interior needles is then undertaken, with the remaining 25% of total activity implanted. This is where the treatment-planning computer is particularly important because it allows another opportunity to ensure the dosimetric quality of the implant. In addition, one can often test new dose distributions by varying needle location to best fit the unique characteristics of the implant to this point. Usually 4–7 interior needles are used, located at least 5 mm away from the urethra. The purpose of the interior seeds is to adequately cover the base and apex and not necessarily to provide a high amount of radiation to the center. For centers that are beginning an implant program, we advocate internal needle placement in a U-shape around the urethra. As one gains confidence in the procedure and the use of intraoperative treatment planning software, the inner needle distribution can be more variable and continue to fulfill the planned dose constraints (Fig. 1). In addition, at this point the intraoperative dosimetry system may be used to rationalize the use of fewer seeds than originally suggested by the nomogram. It is important to always place at least one seed at the apex and at the base, regardless of what the intraoperative software suggests to ensure adequate 30-day postimplant dosimetry.[12–15]

Patients with biopsy-confirmed seminal vesicle involvement and negative nodal involvement should have vesicles implanted. Deposition of seeds is accomplished through the peripheral needles or through 4–5 additional needles that are placed in the seminal vesicles after removal of the interior ones. The seeds are placed in the anterior and posterior walls to ensure that the prescription dose cloud covers at least the proximal half of the seminal vesicles.[16,17]

When all seeds are implanted, a dynamic cystogram under fluoroscopy is performed to exclude the possibility of seeds

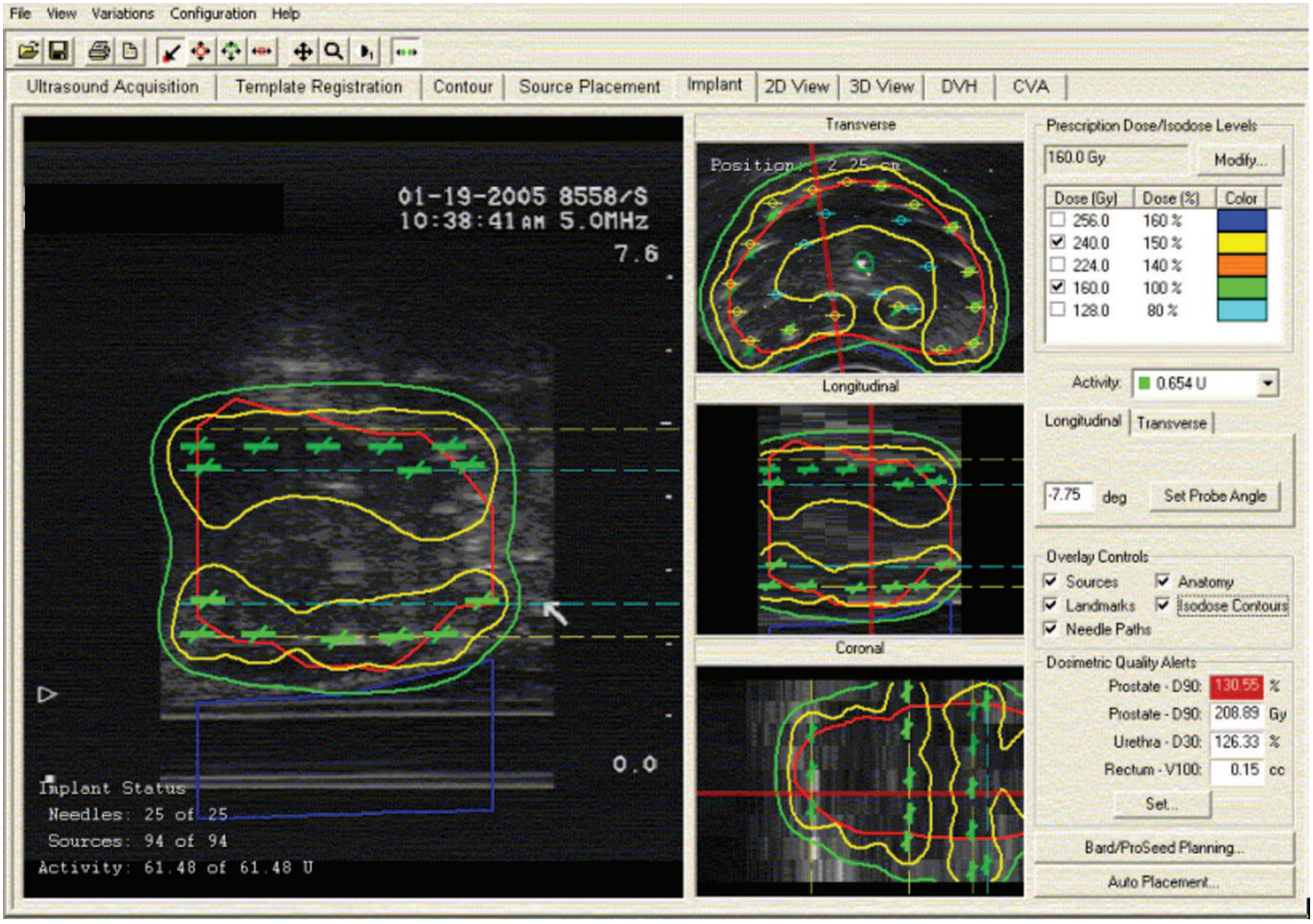

FIGURE 1. Intraoperative treatment plan for a peripherally weighted low dose rate implant using Variseed 7.1 (Varian, Palo Alto, CA).

placed in the bladder or in the urethra. If present, these can be removed before the patient is taken to the recovery room.

PREPLAN

After the introduction of image-guided seed deposition with the use of axial transrectal ultrasound by Holm, physicians at the Seattle Prostate Institute refined this original technique by developing the preplanned method of prostate brachytherapy in the mid-1980s.[18] According to this method, a plan is created by the physics staff a few days before the implant by using the transverse transrectal ultrasound images captured in the office. The patient is similarly positioned in the operating room to duplicate the preplan, the predetermined coordinates are identified, and preloaded needles are then placed.

The plan starts with a volume study of the prostate in the urologist's office, where transverse images are generated at 5-mm intervals and carefully outlined with a light pen. Then each of the images is entered into the treatment planning computer software that generates a three-dimensional model of the gland and calculates the position of each seed into the prostate with dose designation. Finally, this plan is used in the operating room where physicians attempt to put the patient in the same position as when the preplan was created, by meticulous duplication of external set-up parameters such as hip and knee angles. Needles are preloaded with spacers and are then inserted by the use of a template through the perineum in the prostate. The transrectal ultrasound probe is not used to direct the seed placement but is used to assist in the re-creation of the preplan and assure that the needles are positioned in the predetermined locations.[19,20]

The implant begins anteriorly and proceeds posteriorly. Each needle is inserted into its preplanned grid location and then is carefully withdrawn, keeping the obturator stationary for the entire row of alternated seeds to be placed in the predetermined position. Loose seeds can also be placed using the Mick applicator, should the clinician identify a potential deficit not encountered by the preplanning team.

When all needles are inserted, a cystoscopy is performed to identify whether any needle was placed in the urethra or bladder. If so, the preloaded strand is removed and reloaded in the needle for repeated insertion.

In their early implantations the Seattle group used uniform placing of seeds throughout the prostate. Later,

peripheral deposition was used to avoid high doses to the central part of the gland. In addition, the initial ultrasound probes did not allow for a biplanar view, which meant that when the technique originated, only the transverse image was available, creating the need to rely upon the preplan and identification of a fixed base point from which to implant all sources. The introduction of the biplanar probe improved identification of the apex and base, resulting in improved coverage of the ends of the gland.[21]

OTHER TECHNIQUES

Magnetic-Resonance Imaging Guided

In this technique real-time magnetic resonance (MR) imaging is used for performing prostate seed implantation and is described by D'Amico et al[22,23] as follows. A MR-compatible perineal template is fixed to the MR imaging couch in a way that allows movement of the template in 3 dimensions and an MR imaging unit are used. A rectal obturator is passed through the template and fixed. Through the obturator, intrarectal gas is passed, ending in a rubber tube that fills the rectum. Its function is to fix the prostate and to assure that the rectal wall will stay out of the course of the needles. The seeds are loaded in the periphery.

Axial, coronal, and sagittal images at 5-mm intervals using a MR coil are acquired in a 0.5-Tesla magnetic field. The peripheral zone of the prostate (clinical target volume), prostatic urethra, and anterior rectal wall are identified in each axial slice. Each MR-compatible catheter loading is calculated using an algorithm. The preloaded catheters with seeds are inserted through the template and via the perineum into the prostate. Their position is identified in all 3 planes and compared with the expected location according to the treatment plan. The coronal view provides information to the physician about whether the needle has deviated from its straight path. The same process is repeated for all planned catheters.[24,25]

At the end of the procedure, a cystoscopy is performed for assessment of bladder neck integrity and for identification and removal of any seeds placed in the bladder.

Radioimmunoguided

This technique uses the radiolabeled antibody, In-111 capromab pendetide (ProstaScint), specific for prostate-specific membrane antigen to identify regions within the prostate gland where there is a preponderance of tumor. The single-photon emission computed tomography images obtained from the ProstaScint scan are fused to pelvis computed tomography imaging; through this fusion areas of increased uptake are identified, and using a preplanned approach, similar to the one outlined above but crafted to intentionally allow the 150% prescription isodose line to cover the areas of high ProstaScint activity.[26,27]

Robot-Assisted

This technique is in its infancy and has only been described from a feasibility standpoint. Largely because of the relatively few side effects conferred by the procedure in relatively inexperienced hands and the ease with which the technique can be adopted, it is unlikely that this approach will find an indication until its cost has decreased dramatically.[28,29]

TABLE 1. Results of Low-Dose Rate Brachytherapy for Patients With Low-Risk Prostate Cancer

Author	Patient Numbers	Definition	Median Follow-up	Years	Rate (%)
Ellis et al[30]	239 (all risk groups)	ASTRO	47 mo	7	96%
Zelefsky et al[31]	319	ASTRO	63 mo	5	96
Zelefsky et al[32]	1,444	ASTRO	63 mo	8	82
Block et al[33]	118	ASTRO	49 mo	5	94.7
Khaksar et al[34]	146	ASTRO	45 mo	5	96
Guedea et al[35]	241	ASTRO	30 mo	3	93
Stock et al[15]	589	ASTRO	4.2 y	10	94
Prada et al[36]	275	ASTRO	31 mo	5	96
Potters et al[37]	481	ASTRO-Kattan	82 mo	12	89
Sharkey et al[38]	? of 1,707	ASTRO	?	12	89
Joseph et al[39]	? of 667	ASTRO	31 mo	8	84.3
Critz and Levinson[40]	? of 1,469	>0.2	6 y	10	93
Bladou et al[41]	177	ND	29 mo	3	98
Battermann et al[42]	114	ASTRO	48 mo	5	89
D'Amico et al[43]	196	ASTRO	3.9 y	5	95
Sylvester et al[44]	63	2 PSA rises	63 mo	10	89
Kwok et al[45]	41	ASTRO	7 y	5	85
Grimm et al[46]	125	2 PSA rises	81 mo	10	87
Wallner et al[47]	126	>0.5	2.9 y	3	89–91
Martin et al[48]	273	Houston	5 y	12	90
Merrick et al[34]	120	ASTRO	31 mo	5	97

ND, not determined.

PROSTATE-SPECIFIC ANTIGEN (PSA) CONTROL RATES

Low Risk

Patients with low-risk prostate cancer are particularly well suited for low-dose rate brachytherapy. Although various brachytherapy regimens, including implant alone, implant plus hormonal therapy, and combined implant and external beam radiotherapy (EBRT), have been used for patients with low-risk cancers, it has been the consensus of most brachytherapists, as well as the American Brachytherapy Society, that low-dose rate brachytherapy alone is the optimal regimen to maximize cancer control while minimizing morbidity (Table 1). Of the 7 series listed in the table with 10 years of follow-up, the rate of durable biochemical control ranges from 87% to 94%.

Intermediate Risk

For patients with intermediate-risk prostate cancer, generally those with a Gleason score of 7, a PSA value 10 or a palpable stage T2b tumor, many practitioners have added either hormonal therapy or EBRT to confer a high cure rate. At Mount Sinai, the following treatment algorithm has evolved. The preferred treatment currently for intermediate-risk prostate cancer is the combination of neoadjuvant antiandrogen therapy for a duration of 3 months, followed by a prostate seed implant to a full dose. This regimen has been shown to improve outcomes compared with those with brachytherapy alone. An alternative option is to combine a partial-dose brachytherapy implant with supplemental EBRT to 45 Gy. Generally, at approximately 7 or more years, the reported biochemical control rate ranges from approximately 70% to 95% (Table 2). There is certainly also heterogeneity in this group of patients based upon the definitions of intermediate-risk used as well as the volume of cancer as determined by a pretreatment biopsy. With longer follow-up and improved staging (such as using percent of biopsy involved with the tumor), a brachytherapist should be able to further identify patients with more advanced intermediate-risk features and determine more precisely which patients would benefit from the addition of EBRT to the prostate only or to the pelvis with or without concurrent adjuvant hormone therapy. This understanding has the potential to bring all treated series to an 80%–90% freedom from biochemical failure rate at and beyond 5 years minimum follow-up.

High Risk

From the early inception of treating prostate cancer with brachytherapy, it became known that patients with high-risk disease faired poorly when treated with a seed implant alone.[53,54] This knowledge led to the practice of combining brachytherapy with EBRT to treat these patients. This approach has resulted in excellent disease control rates (Table 3). At Mount Sinai, using an approach that involves 9 months of hormonal therapy, 103-Pa brachytherapy and external beam irradiation, the 7-year biochemical control rate was 83% for 360 patients with high-risk prostate cancer.[54] Dattoli et al[55] reported on 243 patients with high-risk disease treated with combination therapy and showed an 80% biochemical control rate at 13 years. These excellent rates compare favorably to those with radical prostatectomy, especially when one focuses on the subset of patients with high-grade tumors (Gleason score 8–10). At Mount Sinai patients with a Gleason score of 8–10 had a 77.5% freedom from PSA failure (FFPF) rate at 7 years.[54] This appears to be superior to the 10% to 39% rate found after radical prostatectomy alone.[58,59]

IMPORTANCE OF IMPLANT QUALITY

When transperineal ultrasound–guided prostate brachytherapy began to be implemented into clinical practice in the late 1980s, limited data were available to guide physicians in determining the appropriate prescription dose for I-125 seed implants. At that time, the commonly used prescription dose of 160 Gy was derived from the original work done by Hilaris at Memorial Sloan Kettering Cancer Center, where 160 Gy was chosen as the dose to be delivered by permanent I-125 seed prostate implants.[60] Although this dose

TABLE 2. Patients With Intermediate-Risk Prostate Cancer Treated With Prostate Brachytherapy

Author	Patient Numbers	Definition	Median Follow-up	Years	Rate (%)
Ellis et al[30]		ASTRO	47 mo	7	87
Zelefsky et al[31]	47	ASTRO	63 mo	5	89
Zelefsky et al[32]	960	ASTRO	63 mo	8	70
Khaksar et al[34]	111	ASTRO	45 mo	5	89
Guedea et al[35]	119	ASTRO	30 mo	3	88
Stock et al[15]	318	ASTRO	4.2 yr	10	89.5
Potters et al[37]	554	ASTRO	96 mo	12	78
Sharkey et al[38]	? of 1,707	ASTRO	?	12	89
Joseph et al[39]	? of 667	ASTRO	31 mo	8	73.9
Critz and Levinson[40]	? of 1,469	>0.2	6 y	10	80
Battermann et al[42]	114	ASTRO	48 mo	5	75
Sylvester et al[44]	92	2 PSA rises	63 mo	10	77
Koutrouvelis et al[50]	68	ASTRO	4.5 y	5	95
Kwok et al[45]	33	ASTRO	7 y	5	63
Merrick et al[51] (Gleason 7)	273	ASTRO	4.7 y	8	94.8

TABLE 3. Series of Patients With High-Risk Prostate Cancer Treated With Brachytherapy in Addition to Hormone Therapy and/or EBRT

Author	Patient Numbers	Definition	Median Follow-up	Years	Rate (%)
Ellis et al[30]		ASTRO	47 mo	7	72.5
Dattoli et al[55]	243	>0.2 mg/mL	8.5 y	13	81
Merrick et al[56]	204	>0.4 mg/mL	7 y	10	86.6
Zelefsky et al[31]	192	ASTRO	63 mo	8	48
Khaksar et al[34]	43	ASTRO	45 mo	5	93
Guedea et al[35]	30	ASTRO	30 mo	3	81
Stock et al[15]	360	ASTRO	4.25 y	7	83
Copp et al[57]	93	ASTRO	45 mo	4	77
Potters et al[37]	418	ASTRO	82 mo	12	63
Sharkey et al[38]	? of 1,707	ASTRO	?	12	88
Joseph et al[39]	? of 667	ASTRO	31 mo	8	52.6
Critz and Levinson[40]	? of 1,469	>0.2 mg/mL	6 y	10	61
Battermann et al[42]	114	ASTRO	48 mo	5	54
Sylvester et al[44]	77	2 PSA rises	63 mo	10	47
Koutrouvelis et al[50]	280	ASTRO	4.5 y	5	81
Kwok et al[45]	28	ASTRO	7 y	5	24

was empirically derived, it was felt to be equivalent to a dose of 67 Gy of EBRT delivered over 7 weeks based on time–dose factor calculations.[61,62] This dose was selected at a time when it was believed that doses of 65–70 Gy of EBRT provided excellent local control based on the subsequent digital rectal examination evaluations.[63–65]

There were significant problems underlying the assumptions that led to the dose choice of 160 Gy for prostate implants. We now know that traditional doses of 65–70 Gy of EBRT have not achieved the high rate of local control as previously reported using digital rectal examination, based on high reported PSA failure rates and positive post-treatment biopsy results after EBRT.[66–68] The assumption that the doses calculated from the original Memorial Sloan Kettering Cancer Center prostate implant experience using a matched peripheral dose technique were the actual doses received by the prostate is questionable, because the isodose lines calculated around the seeds could not be correlated to the actual position of the prostate gland. Finally, the assumption that 160 Gy of I-125 is biologically equivalent to 70 Gy of external beam irradiation based on time–dose factor calculations is now known to probably not be true, based on the more commonly used biologically effective dose (BED) calculations.[69]

Mount Sinai reported the first dose-response analysis for permanent prostate brachytherapy in the modern PSA era. The parameter chosen to measure dose was the dose to 90% of the prostate gland or D90, which was derived from the 1-month postimplant dosimetric computed tomography–based dose volume histogram. This analysis showed a clear dose response, with improved biochemical control associated with increased dose. When patients were separated into 2 groups—those with D90 values less than 140 Gy and those with D90 values ≥140 Gy—there was a large difference in biochemical control rates (68% vs 92% at 4 years, $P = 0.02$) favoring the higher doses. In multivariate analysis, dose was the most significant predictor of outcome.[70] Higher prostate doses were also found to affect local control defined by a negative post-treatment prostate biopsy. Patients with postimplant D90 values for I-125 of <120 Gy had a negative biopsy rate of 69% compared with 96% in those with a D90 values >180 Gy. Patients treated with Pd-103 showed a similar effect. D90 values <80 Gy were associated with a 79% negative biopsy rate compared with 0% positive biopsy with D90 values >120 Gy.[71]

Other investigators have validated this dose-response relationship. Potters et al[72] found a dose-response relationship for I-125 implants and Pd-103 implants combined with EBRT. The dose parameter that demonstrated the response was the D90. With a median follow-up of 30 months, a D90 dose-response cutoff value >90% of the prescribed dose was identified. Prostate implants with a D90 dose <90% had an 80.4% 4-year PSA-relapse free survival, whereas those with a D90 dose >90% had a 92.4% 4-year PSA-relapse free survival ($P = 0.001$). No cutoff value was found for the V100 and D100 dose that predicted for PSA failure. Wallner et al[47] in a randomized trial testing the effect of Pd-103 versus I-125 in the treatment of low-risk prostate cancer found a dose-response relationship. Those patients receiving a D90 >100% of the prescription dose had a 98% freedom from PSA failure versus 82% for those with D90 <100%.

Zelefsky et al[32] reported on a multi-institutional analysis of patients treated with brachytherapy monotherapy. Of these patients, 1,831 (68%) were treated with I-125 seed implantation, and median follow-up was 63 months. Among patients with D90 values >130 Gy, the 8-year PSA relapse-free survival was 93% compared with 76% for those with lower D90 dose levels ($P < 0.001$). When a multivariate analysis was performed on patients with available postimplantation dosimetric information, D90 emerged as a significant predictor of biochemical outcome ($P = 0.01$).

It has often been difficult to compare different types of brachytherapy regimens in terms of dose because different isotopes with different prescription doses, half-lives, and dose rates have been used. In addition, some regimens add external beam irradiation, with very different fractionation schemes into the mix. To compare these different regimens, Stock et al[69] converted doses from different isotopes and regimens into a single value for each patient by using BED equations based on the D90 values and external beam doses and fractionations. BEDs had a significant impact on PSA failure rates. There was a significant improvement in FFPF rates with increasing BEDs. The 10-year FFPF for the BED groups <100, >100–120, >120 – 140, >140–160, >160–180, >180–200, and >200 were 46%, 68%, 81%, 85.5%, 90%, 90%, and 92%, respectively ($P < 0.0001$). Dichotomizing the data on the basis of the above findings into 2 BED groups—<150 (169 patients) and >150 (1,152 patients)—revealed FFPF rates at 10 years of 69% and 91%, respectively ($P < 0.0001$). In addition, in multivariate analysis, BED was the most significant factor to affect biochemical failure rates. Stone et al[74] reported outcomes from a multi-institutional (6 centers) data set. This analysis of 3,928 patients treated with brachytherapy and with postimplant dosimetry demonstrated a dose-response relationship using BED equations. Patients were divided into dose groups on the basis of D90 values. There were 3 dose groups: low-dose (<140 BED), intermediate-dose (140–200 BED), and high-dose (>200 BED). There was a dose-response seen within disease risk groups. Using the American Society for Therapeutic Radiation Oncology (ASTRO) definition of PSA failure, 10-year PSA disease-free survival rates for low-risk patients were 69.8%, 86%, and 88.1% for low-, intermediate-and high-dose groups, respectively ($P < 0.0001$). For intermediate-risk patients, the rates were 52.9%, 74%, and 94.3% for low-, intermediate-, and high-dose groups, respectively ($P < 0.0001$). For high-risk patients, the rates were 19.2%, 61.8%, and 90%, respectively ($P < 0.0001$).

DISTANT METASTASES AND CANCER-SPECIFIC SURVIVAL

Local disease control should be achieved with brachytherapy in most patients with prostate cancer. The data from several studies indicate that it can, but only under certain conditions and if specific treatment targets are met. Treatment failure in the patients with prostate cancer has typically been determined by a rising PSA value or cause-specific mortality. Local control, however, has been determined by digital rectal examination and by prostate biopsy. The former is not typically used anymore because of its inaccuracy. The latter, although controversial as relating to the true significance of a postimplant "positive biopsy," has been more thoroughly investigated. The 2 major controversial issues that persist are the pathologic interpretation of the tissue in cases of "indeterminate results" and the long-term significance of positive biopsy results.

Several investigators have reported their postimplant biopsy results in 3 classifications: negative, positive, and indeterminate.[75,76] Crook[76] found that the proportion of indeterminate biopsies decreased over time from 33% at 1 year to 7% by year 4. Histologically, these patients have residual prostate cancer in the specimens with "marked radiation effect." Whereas most have considered these classifications nonviable, until recently there have been no long-term studies that have followed these patients and documented eradication or progression of the disease.

Stone et al[77] recently reported the long-term patterns of local failure in 508 men who agreed to have a prostate biopsy a minimum of 2 years postimplant. The overall local failure by biopsy was 7.7%. Patients with higher radiation doses had a local failure rate of 3.6% versus 12.5% for low-risk ($P < 0.001$), 5.7% versus 14% for intermediate-risk ($P = 0.039$), and 4.5% versus 18.8% for high-risk ($P = 0.035$) disease. For the individual isotopes, to constitute a high dose, a D90 of at least 160 Gy for I-125 or 124 Gy for Pd-103 or the use of combination therapy had to be delivered. In patients receiving combination therapy, the higher-dose patients received 45 Gy of EBRT and a minimum of 100 Gy of Pd-103. In addition, hormone therapy appeared to further improve the biopsy results from 11.8% to 3.3% positive in patients with both intermediate- and high-risk disease who received the higher doses ($P = 0.087$).

In this study, routine prostate biopsies were offered at 2 years post-therapy regardless of disease status and 10% (52 of 508) had positive biopsy results. In 44% of these patients, results eventually reverted to negative. This finding is in contrast to the report of Prestidge et al[75] of 22% positive or "indeterminate" results after 1 year.[75] Almost all of the positive 2-year biopsy results in our patients demonstrated the same results. Whereas Prestidge et al believed that most of these positive results would "clear" with time (although this was not actually demonstrated in their study), our data suggest that this happens less than half the time. These data can present a conundrum for the urologist who elects to perform a biopsy on patients 2 years after brachytherapy. Although biopsies are not routinely performed in clinical practice, the decision to perform a biopsy after brachytherapy may be made if the patient experiences a rise in PSA value after reaching a nadir. Unfortunately, up to 31% of patients can experience a temporary rise in PSA value after brachytherapy, which is not associated with either local or systemic disease progression.[78,79] Our suggestion would be to observe these patients and not perform a biopsy if the D90 is >100%. If the patient experiences several consecutive PSA elevations, a biopsy may become necessary.

The 10-year FFPF was 80% if the biopsy results were negative versus 27.3% if results of the final biopsy were positive ($P < 0.0001$). The significance of positive postirradiation biopsy results has been controversial in the past. The study of Stone et al,[77] with long follow-up and repeat biopsies, confirms that persistently positive biopsy results lead to disease progression. In fact, positive biopsy results were 18.5 times more likely to result in deaths from prostate cancer than negative results ($P < 0.0001$).

Stock et al[78] has also described the effect of the BED on biopsy outcomes. The D90 values were converted into a BED for each isotope, which was summed with the BED of EBRT

TABLE 4. Postimplant Biopsy Results of Several Representatives Series

Study	Number	Positive Biopsy Results (%)	Implant Dose Related	Positive Biopsy Results for Good- vs Poor-Quality Implant
Prestige et al[75]	402	20	N/A	
Kuban et al[103]	55	18	N/A	
Vijverberg et al[80]	52	50	Yes	20 vs 80
Stone et al[77]	508	7	Yes	4.4 vs 16.2
Stock et al[78]	432	7	Yes	4 vs 13

N/A, not applicable.

for patients receiving combination therapy. This allowed comparisons of treatment factors to one dose equivalent when factors influencing the biopsy outcomes were evaluated. Multivariate analysis revealed that only hormone therapy ($P = 0.05$) and BED ($P = 0.006$) had significant influences on biopsy results. Table 4 lists the published studies detailing biopsy results after brachytherapy.

TREATMENT-RELATED SIDE EFFECTS

The side effect profile for most men who undergo brachytherapy monotherapy is relatively benign. The majority of patients will be able to urinate immediately after the procedure. Urinary obstruction does occur at a rate of approximately 5% although its occurrence mostly depends on the patient's preimplant urinary symptoms.[81] Complete retention is related to obstructive uropathy and correlates with elevated American Urology Association scores.[82] Immediately after the implant the patient may notice blood in the urine, which should clear after the first few voiding attempts. If the patient attempts sexual relations in the immediate postbrachytherapy period, it is possible that a seed may be ejaculated; therefore, it is recommended that patients use a condom within the first several months after the procedure. Hematospermia may also occur for some time after the implant.[83]

Within a few weeks most men will begin to experience urinary symptoms. The severity and nature of these symptoms can vary dramatically although the majority of men will have increased frequency and urgency. If these symptoms are troublesome, α-blockers and anti-inflammatory medications such as ibuprofen typically offer some relief. Burning or dysuria can be managed by urinary analgesics (Pyridium) and by decreasing acid in the diet. These symptoms may take 6 months to resolve.[84,85]

Rectal symptoms may also occur and typically accompany the urinary symptoms. Presumably, inflammation in the prostate is causing inflammation to the rectal mucosa. Constipation can exacerbate the discomfort; therefore, a bulk-producing laxative and use of anti-inflammatory medication are often recommended. Minor proctitis occurs in approximately 10% to 20% of patients and is dose and technique dependent.[86,87] It is best managed conservatively also with a bulk-producing laxative without further intervention. If it persists, a steroid-containing suppository can be added. Aggressive management by rectal wall biopsy or fulguration (or other caustic therapy) should be strictly avoided as this therapy has been reported to be one of the main treatments associated with ulcer and fistula formation. The rectal mucosa next to the prostate often gets a very high dose of radiation, leading to fibrosis and decreased vascularity of the submucosa and rectal wall. Laser or cautery therapy or aggressive rectal examination can result in permanent injury, leading to a prostate-rectal fistula. Subsequent management of this complication may include early bowel diversion, hyperbaric oxygen treatment, urinary diversion with a suprapubic tube and resection of the affected tissues with construction of a neobladder and a coloanal reanastomosis. In some cases, a small fistula has been successfully managed with urethral catheter drainage. This is a rare complication, which has occurred in approximately 1 in 1,000 patients.[88,89]

Erectile dysfunction is a common consequence of aging and had been very difficult to quantify accurately in patients sent for radiotherapy because this group has, for the most part, been older than 65 years. Commonly quoted incidence rates of erectile dysfunction after brachytherapy range from 20% to 40%, usually several years after the therapy.[90–92] A recent study from Mount Sinai of 131 men with optimal erectile function before prostate brachytherapy and followed for a minimum of 7 years showed that the patient's age significantly influenced potency preservation. A 50-year-old man had a 92% likelihood of maintaining erectile function compared with 64% and 58% for the patient in his 60s or 70s, respectively.[93] These age-related data are similar to those found for patients undergoing radical prostatectomy.

There is a risk of secondary malignancy risk with all exposures to radiotherapy. This is a stochastic phenomenon, which means that the incidence appears to be random. The pediatric population has the greatest risk for a clinically meaningful increase in secondary malignancies after exposure to radiotherapy. In the population older than 40 years, it is possible to measure, on the basis of large epidemiologic studies, an increased malignancy rate among those exposed to radiotherapy. Regarding brachytherapy specifically, a single institution data set found that among 348 patients followed for a median of 10.8 years an excess of 35 cancers per 10,000 patients was seen after the brachytherapy procedure.[94] Although this incidence is statistically significant, the authors felt that the incidence had little clinical meaning. In addition, the risk of bladder and colorectal cancer was 1.6% in the brachytherapy alone group versus 5.8% in the brachytherapy plus supplemental radiotherapy group ($P = 0.06$) with a trend toward significance. A larger Surveillance Epidemiology and End Results (SEER)-Medicare database search of men with the diagnosis of prostate cancer from 1973 to 1999 identified

297,069 men, of whom 29,529 had a second cancer diagnosis.[95] The study was limited to men who were alive 5 years after the initial diagnosis and showed that of the final study population of 140,767 men, those receiving EBRT had a small but statistically significant risk of a secondary cancer whereas those treated with an implant alone or in combination with EBRT radiotherapy did not have an increased risk of secondary cancers. Another interesting recent study, also from the SEER database, looked at men with the diagnosis of prostate cancer from 1973 to 2001 as their first carcinoma, identifying 237,772 patients.[96] These were divided into 3 groups, those who had surgery, radiotherapy, or conservative management. It was found that men having the surgery had the lowest incidence of rectal cancer, and men with conservative management had the highest incidence of rectal cancer. In an interesting letter to the editor regarding the article, David Brenner[97] put forth evidence that there is a known higher incidence of smoking among men conservatively treated for prostate cancer in Canada and that furthermore this incidence in smoking could be the causal factor behind the increased incidence in rectal cancer in the untreated group. In addition, he described a possible association between a low testosterone environment and a causal association to the onset of rectal cancer. On the basis of the work done so far, it is possible to conclude that the rate of secondary cancers after brachytherapy is very small and clinically insignificant.

GENETIC PREDICTORS OF SIDE EFFECTS

A compelling body of work regarding the potential of genetic testing to predict possible side effects is now being recognized. In a series of studies in breast cancer and prostate cancer, a relationship has been found between the incidence of radiotherapy-induced fibrosis and the expression of the ataxia-telangiectasia mutation (*ATM*) gene.[98] In breast cancer, heterozygosity in the *ATM* gene manifests as telangiectatic skin changes and subcutaneous fibrosis.[99] In men treated with I-125 brachytherapy for prostate cancer, the same profibrotic mechanisms are related to erectile dysfunction, late rectal bleeding, and urinary bother. Among men treated with prostate brachytherapy, the initial results of an association between erectile dysfunction and *ATM* status of the pilot study of 37 men has been validated in a new group of 98 additional men.[100,101] In addition, of note, a larger study of 108 men whose *ATM* status was clarified showed that in the low dose range of rectal exposure those men with *ATM* heterozygosity accounted for almost all of those who had late proctitis.[102] These initial findings have led to significant associations in other important molecules and the late effects of radiotherapy. In the future, as the cost of genetic screening continues to fall, one may be able to predict with a reasonable degree of certainty not only whether a patient will experience any side effect from brachytherapy or EBRT but possibly also which side effect and its severity. This information will certainly add to the armamentarium of knowledge that a counseling physician will be able to give to the patient to facilitate the best possible outcome of prostate cancer treatment.

CONCLUSION

Prostate brachytherapy is an excellent treatment modality for localized prostate cancer. The major side effects are temporary urinary symptoms. In the future, we will most probably be able to better inform patients about their specific risks of side effects, thereby decreasing substantially the influence of any given physician's therapeutic bias in the face of several reportedly equivalent therapies.

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CLINICAL INVESTIGATION **Prostate**

CHANGING THE PATTERNS OF FAILURE FOR HIGH-RISK PROSTATE CANCER PATIENTS BY OPTIMIZING LOCAL CONTROL

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Purpose: Standard therapies for high-risk prostate cancer have resulted in suboptimal outcomes with both local and distant failures. Prostate-specific antigen (PSA) and distant metastases rates as well as biopsy outcomes are reported after a regimen of trimodality therapy with hormonal, radioactive seed, and external beam radiation therapy to demonstrate how patterns of failure are changed when local control is optimized.

Methods and Materials: From 1994 to 2003, a total of 360 patients with high-risk prostate cancer were treated with trimodality therapy. Patients were defined as being at high risk if they possessed at least one of the following high-risk features: Gleason score 8 to 10, PSA >20, clinical stage t2c to t3, or two or more intermediate risk features: Gleason score 7, PSA >10 to 20, or stage t2b. Patients were followed for a median of 4.25 years (range, 2 to 10 years).

Results: The actuarial 7-year freedom from PSA failure and freedom from distant metastases (FFDM) rates were 83% and 89% respectively. Patients ($n = 51$) developing PSA failure exhibited aggressive disease behavior with short PSA doubling times (median, 5 months) and a 7-year freedom from distant metastases rate of 48%. Local control was high. The last posttreatment biopsy results were negative in 97% of cases (68 of 70 patients). In multivariate analysis, only PSA >20 predicted biochemical failure ($p = 0.04$), and only seminal vesicle status predicted developing distant failure ($p = 0.01$).

Conclusions: Trimodality therapy results in excellent local control that alters patterns of failure, resulting in similar actuarial biochemical and distant failure rates. Most failures appear to be distant and exhibit biologically aggressive behavior. © **2006 Elsevier Inc.**

Prostate cancer, Brachytherapy, High-risk, distant metastases, Local control.

INTRODUCTION

Historically, treatment for locally advanced or high-risk prostate cancer has resulted in less than optimal rates of disease control (1–5). Explanations for these outcomes have focused on the presence of microscopic disseminated disease at presentation. For this reason, little attention has been focused on optimizing local control and more on developing new systemic approaches. The patterns of failure following standard treatment for high-risk prostate cancer reveal a large component of local recurrence in addition to distant spread of disease (5–7). The reason that local recurrence continues to be a problem stems from both the inability of radical prostatectomy to remove all known disease because of extracapsular and seminal vesicle spread, and the failure of external beam radiation therapy to eradicate all known disease, primarily because of inadequate dose delivery. To address this problem, we developed a treatment approach for high-risk prostate cancer that was designed to optimize local control by using brachytherapy, external beam radiation therapy, and neoadjuvant and concurrent hormonal therapy (8). The goal was to deliver a higher dose to the prostate, seminal vesicles and immediate surrounding tissue than could be delivered with external beam radiation therapy alone (9) in combination with the potential synergism of hormonal therapy. To delineate the patterns of failure after this regimen, biochemical recurrence and distant metastases rates as well as posttreatment biopsy results were reported. Because the source of a biochemical failure is often elusive, the relationship between biochemical failure and distant metastases was explored to shed light on this problem. In addition, factors that affect both PSA failure and distant metastases rates were analyzed to determine those patients at greatest risk for failure. Finally, an examination of the natural history of patients experiencing prostate-specific antigen (PSA) failure was made after the above-mentioned combined modality therapy to demonstrate how patterns of failure change when local control is optimized.

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Table 1. Presenting clinical characteristics of study patients

Factor	No. of patients	%
PSA		
≤10	150	42
>10–20	121	34
>20	89	24
Gleason score		
≤6	60	17
7	176	49
8–10	124	34
Clinical stage		
T1c	59	16
T2a	32	9
T2b	144	40
T2c	95	27
T3a–c	30	8

Abbreviation: PSA = prostate-specific antigen.

METHODS AND MATERIALS

Patient selection

From 1994 to 2003, a total of 360 patients with high-risk prostate cancer were treated with combined modality therapy consisting of brachytherapy, external beam radiation therapy, and hormonal therapy. Patients were defined as high risk if they possessed at least one of the following high-risk features: Gleason score 8 to 10, PSA >20 ng/ml, clinical stage t2c to t3, or two or more of the following intermediate risk features: Gleason score 7, PSA >10 to 20 ng/ml, or stage t2b. No patient had radiologic or pathologic evidence of metastatic disease. Patients were staged using the 1992 American Joint Committee on Cancer (AJCC) staging system (10). All patients had bone scans and computed tomography (CT) scans of the pelvis, which were negative for metastatic disease. The presenting disease characteristics of these patients are summarized in Table 1. Bilateral ultrasound guided seminal vesicle biopsies (6) were performed at the discretion of the referring urologist (11). Seminal vesicle biopsies were performed in 216 patients; results were positive in 45 patients and negative in 171 patients. Laparoscopic lymph node dissection (LPLND) was performed in 68 patients; this procedure was performed in patients with positive seminal vesicle biopsy or at the discretion of the referring urologist (11). Node-positive patients were not included in this analysis.

Treatment

The treatment regimen began with hormonal therapy with an LHRH agonist alone (25%) or with an anti-andgrogen (75%) for 3 months. This was followed by a brachytherapy implant to the prostate (± seminal vesicles) using the isotopes Pd-103 in 356 patients and I-125 in 4 patients. All implants were performed using a real-time ultrasound-guided technique. Details of this procedure have been previously described (12, 13). In 1994, the prescription dose for Pd-103 (all doses were converted to the guidelines of the National Institutes for Standards and Technology, 1999) started at 62 Gy and was increased to 100 Gy by 1998 (14). The prescription dose for I-125 was set at 120 Gy (Task Group 44). One month postimplant, CT-guided dosimetry revealed D90 (dose to 90% of prostate volume from the dose–volume histogram) values for Pd-103, which ranged from 28 to 157 Gy (median, 99 Gy) and for I-125 from 120 to 150 Gy (median, 128 Gy). Seminal vesicles were implanted in those patients with positive seminal vesicle biopsy results or in those who did not undergo biopsies. This technique has also been previously described (15). Two months after brachytherapy, external beam radiation therapy was delivered to the prostate and seminal vesicles with margins of 1 to 1.5 cm using three-dimensional (3D) conformal or intensity-modulated radiation therapy techniques. The median external beam radiation therapy dose delivered was 45 Gy (39.6–59.6 Gy). Lower implant prescription doses were associated with higher external beam radiation therapy doses (14). Hormonal therapy was continued for a total of 9 months.

Follow-up

Patients were asked to return every 6 months after completion of therapy. Follow-up generally consisted of a digital rectal examination and PSA. Follow-up time was calculated from completion of treatment to date last seen and ranged from 2 to 10 years (median, 4.25 years). CT and/or bone scans were performed in the setting of a rising PSA profile or a symptomatic patient. PSA failure was determined using the definition of the American Society for Therapeutic Radiology and Oncology (16). In patients with a PSA failure, PSA doubling times (DT) were calculated using first-order kinetics. Most patients were placed on hormonal therapy when they were determined to have biochemical failure.

Distant metastases included disease spread to bones, visceral organs, or lymph nodes outside of the pelvis as documented by imaging studies.

Biopsies (8–10 core samples) were recommended at 2 years posttreatment. Biopsy results were read as negative or positive with no indeterminant reading. Repeated biopsies after this point were performed in the event of initial negative biopsy with continued rise in PSA or of initial positive biopsy with no evidence of a rising PSA. The outcomes for the biopsies were based on the last biopsy performed. Of the patients, 59 underwent one biopsy, 10 underwent two biopsies, and 1 patient underwent three.

Statistical analysis

Survival curves were determined using the methods of Kaplan and Meier. Differences in survival rates were calculated using the log-rank test. Multivariate analysis of survival was performed using a Cox regression analysis with the enter model and variables entered at a level of 0.05 and removed at a level of 0.1 (17).

RESULTS

Of the 360 patients, 51 developed PSA failure for an actuarial freedom from PSA failure (FFPF) rate of 83% at 7 years (Fig. 1). The time from completion of treatment to PSA failure ranged from 136 to 2093 days (median, 676 days). Of all PSA failures, 78% occurred within 3 years of completion of treatment. The last PSA values for those without a PSA failure were ≤0.1 ng/ml in 86%, >0.1 to 0.2 ng/ml in 8%, >0.2 to 0.5 ng/ml in 3%, and >0.5 ng/ml in 3%. The last testosterone levels for patients without PSA failure were ≤100 ng/dl in 14%, >100 to 200 ng/dl in 11%, >200 to 800 ng/dl in 73%, and >800 in 2% ng/dl. A total of 20 patients developed distant metastases for an actuarial freedom from development of distant metastases rate (FFDM) at 7 years of 89% (Fig. 2). In patients with PSA failure, the FFDM rate at 7 years was 48% (Fig. 3). The time

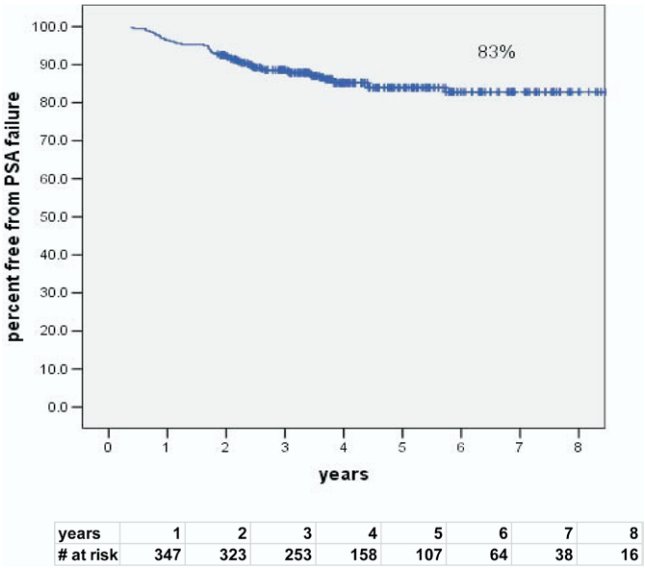

Fig. 1. Actuarial freedom from prostate-specific antigen (PSA) failure.

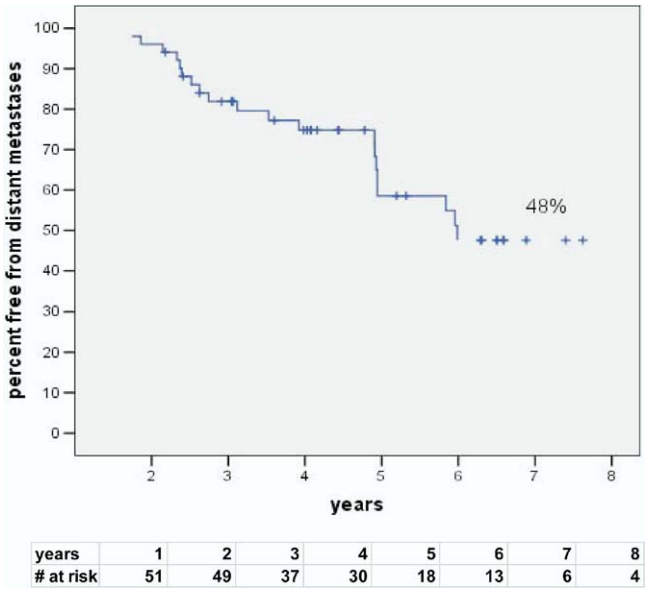

Fig. 3. Risk of developing distant metastases in patients with prostate-specific antigen (PSA) failure.

from PSA failure to the development of distant metastases ranged from 45 to 1711 days (median, 529 days). The PSA DTs for the 51 patients with a PSA failure ranged from 1 to 203 months (median, 5 months). DT were ≤3 months in 38%, >3 to 6 months in 20%, >6 to 10 months in 8%, and >10 months in 34%.

The effect of pretreatment PSA, Gleason score, stage, and seminal vesicle status on both FFPF and FFDM rates were analyzed and are shown in Table 2. Patients with PSA levels >20 ng/ml had a significant higher biochemical failure rate than patients with PSA <20 ng/ml ($p = 0.01$). There was a significant difference in both PSA failure and distant metastases rates among the three seminal vesicle status groups. Further comparisons showed that there were significant differences between positive and negative seminal vesicle biopsy patients in both PSA failure rates ($p = 0.007$) and distant metastases rates ($p = 0.012$). A comparison between positive biopsy patients and those with no biopsy revealed a significant difference in PSA failure ($p = 0.028$) but not in distant metastases rates (0.9). A comparison between negative biopsy patients and those not undergoing biopsy re-

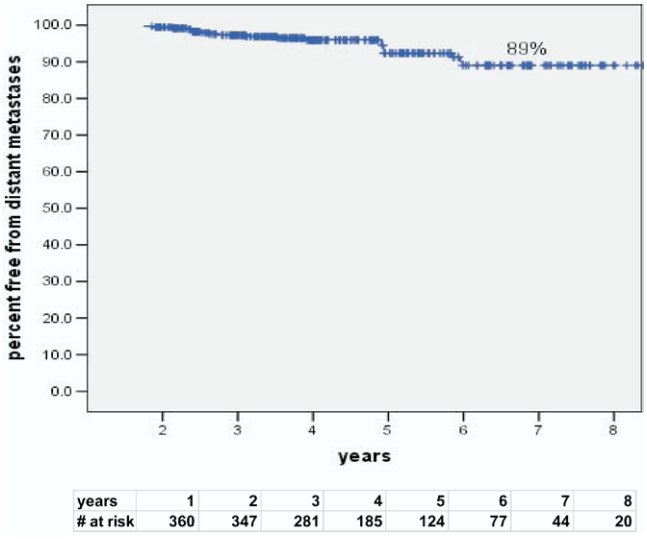

Fig. 2. Actuarial freedom from development of distant metastases.

Table 2. Effect of disease factors on prostate-specific antigen (PSA) failure and distant metastases at 7 years

Factor	FFPF	FFDM
PSA		
≤10	87%	89%
>10–20	87%	88%
>20	72%	89%
p value	0.01	0.74
Gleason score		
≤6	83%	93%
7	86%	90%
8–10	77.5%	85%
p value	0.08	0.5
Clinical stage		
t1c	80%	94%
t2a	78%	78%
t2b	84%	86%
t2c	87%	94%
t3	75%	80%
p value	0.8	0.3
Seminal vesicle status		
Negative	86%	96%
Positive	67%	78%
Not done	85.5%	71%
p value	0.02	0.02

Abbreviations: FFDM = actuarial freedom from development of distant metastases; FFPF = actuarial freedom from prostate-specific antigen failure.

Table 3. Multivariate analyses for prostate-specific antigen (PSA) failure and distant metastases

	PSA failure		Distant metastases	
Factor	*p* value	Exp (B)	*p* value	Exp (B)
PSA	0.04	1.68	0.92	1.04
Gleason score	0.11	1.56	0.31	1.63
Clinical stage	0.78	1.04	0.58	1.15
Seminal vesicle status	0.36	1.15	0.01	2.07
Risk 1	0.95	1.02	0.94	0.95
Risk 2	0.77	1.14	0.35	2.02

Abbreviations: Risk 1 = one high risk vs. two or more intermediate-risk features; Risk 2 = 2 or more high-risk features vs. all other patients.

vealed a significant difference in distant metastases rates ($p = 0.034$) but not in PSA failure rates ($p = 0.65$).

To test whether it is valid to group both patients with two or more intermediate risk features and those with one or more high-risk features into one risk category, these two subgroups were compared. There were no significant differences in FFPF or FFDM rates between these two subsets of patients. Patients included in this study because of two or more intermediate risk features had 7-year FFPF and FFDM rates of 89% and 85%, respectively, compared with 80.5% and 89.5% for patients with one or more high-risk features ($p = 0.1$ and $p = 0.44$, respectively). In addition, to test whether the presence of more than one high-risk feature carried a worse prognosis, a comparison between those patients with two or more high-risk features and all other patients was performed. Univariate analysis revealed that those with two or more high risk features had lower FFPF and FDM rates (74% and 85%, respectively) than all other patients (85.5% and 90%) ($p = 0.005$ and 0.02, respectively).

Multivariate analysis

Cox regression analyses were performed to determine the most important predictors of both PSA failure and distant metastases; the results are summarized in Table 3. Factors were categorized in a similar fashion as they were in the univariate analyses (Table 2). Pretreatment PSA was the only significant predictor of PSA failure ($p = 0.04$) and the seminal vesicle status was the only significant predictor of developing metastatic disease ($p = 0.01$).

Biopsy outcome

Overall 70 patients underwent posttreatment biopsy. Patients experiencing a PSA failure were more likely to consent to a posttreatment biopsy than those not undergoing biopsy. For this reason, the actuarial FFPF rate in patients undergoing biopsy was lower (71% at 7 years) than the rate in those not undergoing biopsy (86.5%) ($p = 0.004$). Overall, 3 patients had positive biopsy results, with 1 patient having a subsequent repeat biopsy that was negative. The results of the last biopsies were negative in 68 patients (97%). The higher PSA failure rate in patients who underwent biopsy translated into a nonsignificant but higher distant metastasis rate of 16% compared with 8% for patients who did not undergo posttreatment biopsy ($p = 0.16$).

DISCUSSION

The high biochemical failure rates observed for high-risk patients after both radical prostatectomy and external beam radiation therapy have often been attributed to the presence of microscopic disseminated disease at presentation (3, 4). What has been overlooked is a large component of local failure that contributes to these biochemical recurrence rates. In an idealized model, with perfect local control, any rise in PSA would signal the presence of nodal or distant metastases. In this case, the biochemical failure rate and distant metastases rates would be very similar, assuming that the study had long enough follow-up. The reason that adequate follow-up is needed is that there exists a lag time between the development of biochemical failure and the clinical manifestation of the associated recurrence. Reported rates of biochemical and distant failure after external beam radiation therapy and radical prostatectomy do not support this idealized model. Most series report distant failure rates that are approximately 40% to 45% lower than the biochemical failure rates (2, 5, 18, 19). With adequate follow-up, the main factor contributing to this difference is a component of local failure. Local recurrence has been reported in 20% to 44% of patients with clinical T3 prostate cancer treated with radical prostatectomy (5, 7). Based on the digital rectal examination, the rate of local failure after radiation therapy alone from the European Organization for Research and Treatment of Cancer (EORTC) and the Radiation Therapy Oncology Group (RTOG) prospective trials range from 15% to 42% (2, 18, 19). Posttreatment biopsies after external beam radiation therapy for high-risk prostate cancer have demonstrated residual disease in up to 62% of cases (6). Local failure has been shown to be a particular problem in high-risk patients. In a study by Zelefsky *et al.*, posttreatment biopsies after intensity-modulated radiation therapy were positive in 13% of low-risk patients, 23% of intermediate-risk patients, and 37% of high-risk patients (20). A discrepancy between biochemical and distant failure rates is caused by the fact that many patients with local recurrence experience a slow progression of their disease and some may not manifest distant failure at all (21). Table 4 shows the biochemical and distant metastases rates for the control arms of four prospective randomized trials testing the affects of adding hormonal therapy to external beam radiation therapy. In these studies, the differences between biochemical and distant failure rates range from 18% to 45% (2, 18, 19, 22).

The approach to high-risk disease reported here was designed to optimize local control. Neoadjuvant and concurrent hormonal therapy was used to offer both cytoreduction and synergistic enhancement to the radiation. Combined brachytherapy and external beam radiation therapy was used to de-

Table 4. Biochemical and distant metastasis rates in external beam radiation therapy randomized trials

Study (Ref.)	Rx arm	No. of patients	Eligibility	FFPF	FFDM
RTOG 85-31 (18)	RT alone	489	T3 or N+	10 yr, 9%	10 yr, 61%
RTOG 86-10 (2)	RT alone	230	Bulky T2–T4	8 yr, 10%	8 yr, 55%
RTOG 92-02 (22)	4 mos HRM + RT	779	T2c–T4	5 yr, 65%	5 yr, 83%
EORTC (19)	RT alone	206	T1–T2, Grade 3 or T3–T4	5 yr, 45%	5 yr, 72%

Abbreviations: EORTC = European Organization for Research and Treatment of Cancer; HRM = hormonal therapy; Rx = treatment; other abbreviations as in Table 2.

liver the highest dose possible to the prostate and at the same time to address extracapsular extension of disease. The doses used with this combined approach have been shown to be greater biologically than those obtained with brachytherapy or external beam radiation therapy alone (9). This approach has resulted in both high rates of biochemical freedom from failure as well as negative posttreatment biopsy outcomes. The 7-year FFBF rate for the whole group was 83% and the last negative biopsy rate was 97%. It must be noted that this high biochemical failure rate may be caused in small part by the fact that 25% of patients at last follow-up still had testosterone levels <200 ng/dl. How this rate would change if and when these levels ris with longer follow-up remains to be seen. Although the sample size of patients biopsied represents only 19% (70/360) of the total study population, it is unlikely that the positive rate would change much if the other 81% of patients underwent biopsy, as this group had a much lower PSA failure rate than the biopsied group. This high local control rate has translated into a similarity between the FFPF rate and FFDM rate at 7 years of 83% and 89% respectively. Although the gap between biochemical and distant failure rates has been reduced, how this will affect overall survival is still not clear. These results compare favorably to results from RTOG 9413, which tested the role of whole-pelvis RT given in conjunction with androgen suppression for high-risk prostate cancer (23). Whether the addition of pelvic RT to this regimen would have changed the reported outcomes is not known.

These results also demonstrate that a relatively short course (9 months) of hormonal therapy, when combined with dose escalation, may be all that is needed to improve both local and distant control. This parallels the findings of D'Amico *et al.*, which demonstrated that a short course (6 months) of hormonal therapy in conjunction with external beam radiation therapy could improve both prostate cancer–specific mortality and overall survival over that achieved with external beam radiation therapy alone (24).

This increased local control has changed the patterns of failure from a mix of local and distant components to one of primarily systemic recurrences. This is evidenced by the natural history of the patients with PSA failure in this study. Failure tends to occur early (78% fail within 3 years of completion of therapy) and are characterized by aggressive PSA DT. ODTsf the patients with PSA failure 66% had DT <10 months and 58% had DT <6 months. Short DT have been shown to predict poor outcomes after radiation therapy and prostatectomy (21, 25). In addition, the median DT of these patients is 5 months, which is less than half of the 13-month median DT reported by Lee *et al.* in a series of patients (low to high risk) with PSA failure after definitive external beam irradiation (26). Most of the PSA failures in the current series go on to develop distant metastases with an actuarial rate of 52% by 7 years. With longer follow-up, this rate will most likely increase. These patients also develop clinical evidence of metastases relatively early (median, 529 days after PSA failure).

Because biochemical recurrence after this regimen is an ominous sign for most patients, it would be desirable to identify which patients are at greatest risk for recurrence. The most significant predictor of biochemical relapse on multivariate analysis was PSA >20 ng/ml. This also supports the conclusion that most patients who experience failure probably have microscopic distant metastases. The most significant predictor of distant metastases on multivariate analysis was a positive seminal vesicle biopsy result. The fact that not undergoing biopsy was also predictive highlights the fact that many high-risk patients harbor seminal vesicle spread that cannot be detected by physical or radiographic examination. Based on this finding, we will attempt to obtain seminal vesicle status by biopsy in more of our high-risk patients. Patients with these poor prognostic factors might be better treated with a longer course of hormonal therapy. Trials of 2 and 3 years of hormonal therapy reported by the RTOG and EORTC, respectively, demonstrate decreases in both biochemical and distant failure with longer duration of hormonal therapy (19, 22). These patients represent a good group to study in trials of neoadjuvant and adjuvant systemic therapies. In addition, based on the results of RTOG 9313, whole-pelvis irradiation may be considered in these patients (23).

CONCLUSION

The combined-modality approach for treatment of high-risk prostate cancer results in excellent local control with a 97% posttreatment outcome of negative biopsy results and an 83% FFBF rate. With this type of excellent local control, actuarial FFPF and FFDN rates are similar at 7 years. This has resulted in a change in the patterns of failure, with a predominance of patients with failure exhibiting evidence of biologically aggressive behavior and development of distant metastases.

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CLINICAL INVESTIGATION **Prostate**

COMBINED MODALITY TREATMENT IN THE MANAGEMENT OF HIGH-RISK PROSTATE CANCER

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Purpose: The efficacy of a multimodality protocol using neoadjuvant and concomitant hormonal therapy, brachytherapy, and three-dimensional conformal external beam radiotherapy (RT) in high-risk prostate cancer was evaluated using biochemical outcomes and posttreatment biopsy results.

Methods and Materials: Between February 1994 and November 1999, 132 high-risk patients were treated with combined hormonal therapy (9 months), permanent radioactive seed brachytherapy, and external beam RT, with follow-up ranging from 36 to 88 months (median, 50 months). The eligibility criteria were any of the following: Gleason score 8–10, initial prostate-specific antigen (PSA) level >20 ng/mL, clinical Stage T2c-T3, or positive seminal vesicle biopsy, or two or more of the following: Gleason score 7, PSA level >10–20 ng/mL, or Stage T2b. Twenty percent of patients had a positive seminal vesicle biopsy before therapy. Negative laparoscopic pelvic lymph node dissections were performed in 44% of patients.

Results: The actuarial overall freedom from PSA failure rate was 86% at 5 years. The freedom from PSA failure rate at 5 years was 97% for those with a Gleason score of ≤6 (35 of 36), 85% for a Gleason score of 7 (50 of 59), and 76% for a Gleason score of 8–10 (28 of 37; $p = 0.03$). A trend was noted toward worse outcomes in seminal vesicle biopsy-positive patients, with a 5-year freedom from PSA failure rate of 74% vs. 89% for all other patients ($p = 0.06$). Posttreatment prostate biopsies were performed in 47 patients and were negative in 96% at the first biopsy and 100% at the last biopsy.

Conclusion: Trimodality therapy with androgen suppression, brachytherapy, and external beam RT for high-risk prostate cancer results in excellent biochemical and pathologically confirmed local control. **© 2004 Elsevier Inc.**

Prostate cancer, Brachytherapy, High risk, Locally advanced, Combined modality.

INTRODUCTION

High-risk prostate cancer has remained a therapeutic challenge despite treatment with aggressive monotherapeutic approaches, including surgery, brachytherapy, and external beam irradiation (EBRT) (1). The 5-year biochemical control rates in these patients have been reported to range from 0% to 50% (2–6), with up to 50% of failures occurring locally (7).

These outcomes have led investigators to use new therapeutic interventions to improve the results for this subset of prostate cancer patients. Many of these new approaches have involved combining available modalities such as hormonal therapy (HT), surgery, brachytherapy, and EBRT (8–10). Advances in RT for high-risk prostate cancer have used combined HT and EBRT, as well as dose escalation. These approaches have improved the outcome, with 5-year biochemical cure rates ranging from 50% and 83% (11–16). Although marked improvement has been made with these novel therapies, local disease control, as judged by prostate biopsy results, remains poor, with failure rates of up to 36% (16–19). The correlation between local recurrence and metastatic disease suggests that improved local control may indeed improve overall survival (20).

To improve local control and biochemical cure rates in this subset of patients, in 1994, we initiated a treatment regimen involving 9 months of androgen suppression, permanent radioactive seed brachytherapy, and EBRT. The following analysis reports the biochemical control rates and posttreatment biopsy results after this regimen in high-risk prostate cancer patients. The effect of disease- and treatment-related prognostic variables on outcomes was also tested to help validate the relative effectiveness of this treatment.

METHODS AND MATERIALS

Patients were defined as high risk if they possessed any one of the following high-risk features: Gleason score 8–10,

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Table 1. Number of patients stratified by clinical stage, PSA level, and Gleason score

	T1b	T1c	T2a	T2b	T2c	T3a	T3b	T3c	Total
PSA (ng/mL)									
≤4	0	0	0	0	0	1	0	0	1
>4–10	0	8	8	15	11	5	0	2	49
>10–20	1	11	5	11	9	7	0	0	44
>20–50	0	6	5	3	11	1	0	1	27
>50	0	1	1	3	5	0	1	0	11
Total	1	26	19	32	36	14	1	3	132
Gleason score									
≤6	0	10	8	5	8	5	0	0	36
7	0	12	7	19	15	3	1	2	59
8–10	1	4	4	8	13	6	0	1	37
Total	1	26	19	32	36	14	1	3	132

Abbreviation: PSA = prostate-specific antigen.

initial prostate-specific antigen (PSA) level >20 ng/mL, clinical Stage T2c-T3, or positive seminal vesicle biopsy (n = 89). In addition, patients were classified as high risk if they had two or more of the following intermediate-risk features: Gleason score 7, PSA level >10–20 ng/mL, or Stage T2b (n = 43). To take into account the effects of HT, which can suppress PSA for months after therapy completion, patients were required to have a minimum of 3 years of follow-up from therapy completion to be eligible for this analysis. The decision to treat high-risk patients with combined implantation and EBRT began in 1994. Between March 1994 and October 1999, 154 high-risk patients started treatment with combined implantation and EBRT. Patients excluded from the analysis were those who refused HT (16) and those with follow-up of <3 years (6). The remaining 132 patients constituted the study population for this analysis. A description of the presenting Gleason score and PSA level stratified by clinical stage is given in Table 1. The disease was staged according to the 1992 American Joint Committee on Cancer staging system (21). The presenting PSA levels ranged from 1.3 to 300 ng/mL (median 12, mean 22).

Staging

One pathologist with expertise in prostate cancer diagnosis centrally reviewed the pathologic findings. All patients underwent a bone scan and pelvic CT. Patients with a definitive diagnosis of bone metastases or pelvic adenopathy were excluded. In addition, 121 patients underwent pretreatment seminal vesicle biopsy (SVB). Patients did not undergo SVB either because they refused the procedure or because their urologist was not comfortable performing this type of biopsy. SVB was performed using transrectal ultrasound guidance with three cores taken from each seminal vesicle at the proximal portions of the seminal vesicles (22). Of the 121 patients, 27 (20%) had a positive SVB. Laparoscopic pelvic lymph node dissection (LPLND) was initially performed on all consenting patients, but in the latter years, only on patients with a positive SVB, Gleason score 8–10, or PSA level >20 ng/mL. The LPLND removed lymph nodes from an area bounded by the external iliac vein laterally, the obturator nerve posteriorly, the inguinal ligament distally, and the bifurcation of the common iliac artery proximally. The average number of nodes removed per side was five. In total, 57 patients (43%) underwent LPLND. Node-positive patients were excluded from this analysis.

Treatment

Treatment began with HT. The standard hormonal regimen was a luteinizing hormone-releasing hormone agonist and an antiandrogen. Neoadjuvant HT was given for 3 months before brachytherapy. Seventy-eight percent of patients were treated with a luteinizing hormone-releasing hormone agonist and an antiandrogen agent, 19% with a luteinizing hormone-releasing hormone agonist alone, and 3% with combined antiandrogen therapy (flutamide and finasteride). After 3 months of HT, patients underwent permanent radioactive seed implantation of the prostate (plus the seminal vesicles, if involved) using a real-time ultrasound-guided technique that has been previously described (23). Two months after implantation, patients underwent EBRT of the prostate and seminal vesicles. HT was continued from its onset for a total duration of 9 months. The first 38 patients were treated on an institutional review board–approved Phase II trial. This involved the escalation of the planned dose of ^{103}Pd brachytherapy from 57 to 76 to 90 Gy. Because lower dose implants were used, the EBRT dose was 59.4 Gy. The details of this trial have been previously reported (24). Six patients in this series underwent EBRT at an outside institution and were generally treated to lower EBRT doses. On the basis of the results of this Phase II trial, the decision was made to use a prescription implant dose of 90 Gy with ^{103}Pd and to deliver an EBRT dose of 45 Gy. The practice at our institution has been to modify the supplementary EBRT dose according to variations in the implant dose delivered to 90% of the gland (D_{90}) to optimize the total dose delivered to the prostate. Therefore, 4 patients received a dose of 41.4 Gy, 87 patients received 45 Gy, 1 received 46.8 Gy, 3 received 50.4 Gy, 1 received 54 Gy, 32 received 59.4 Gy, and 1 received 70.2

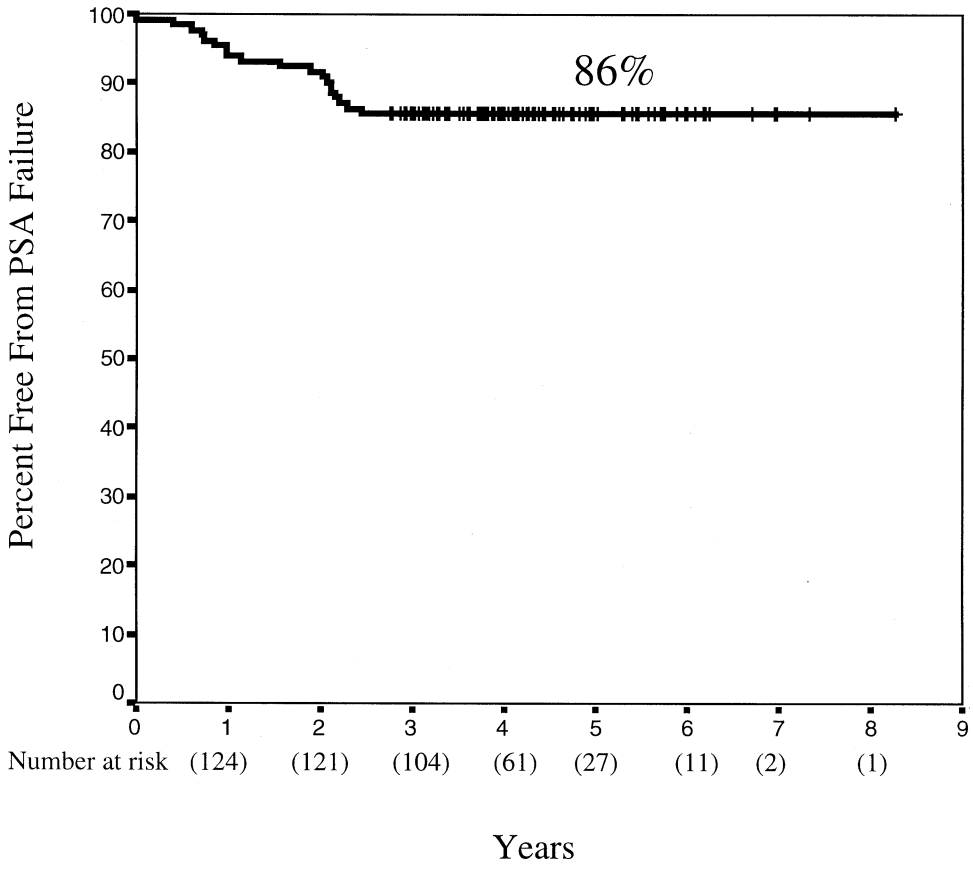

Fig. 1. Five-year actuarial freedom from prostate-specific antigen (PSA) failure rates.

Gy. Overall, 73% received their EBRT at Mount Sinai Hospital and 27% at an outside institution. The isotope most often used was ^{103}Pd (n = 127), although ^{125}I was used in 5 patients, with a prescription dose of 120 Gy. The proximal portions of the seminal vesicles were implanted in those patients who had a positive SVB or in those who did not undergo SVB. Postimplant CT-based dosimetric analysis was performed in patients 4–6 weeks after implantation. The D_{90} to the prostate volume ranged from 28 to 136 Gy (median, 92.2 Gy) for ^{103}Pd implants and from 110 to 150 Gy (median, 125 Gy) for ^{125}I. EBRT was delivered with three-dimensional conformal treatment planning to the prostate and seminal vesicles only. No attempt was made to treat the pelvic lymph nodes.

Follow-up

Patients were followed at 6-month intervals with PSA measurement and, whenever possible, with testosterone measurement. Two years after treatment, transrectal ultrasound-guided prostate biopsies were recommended without regard to disease status. Prostate biopsies were done using 8–12 core samples. In addition, SVBs were also performed after treatment in those patients with a positive SVB before therapy and in those with a rising PSA level. Follow-up from RT completion ranged from 36 to 90 months (median, 50 months). PSA failure was defined using the American Society for Therapeutic Radiology and Oncology definition (25). This definition was selected because it is the most widely used and accepted definition of biochemical failure after RT. The potential limitations of this definition in patients receiving HT include false labeling of PSA failure in a patient whose PSA rises as their testosterone increases. Patients whose PSA values increased as their testosterone levels increased and then began to fall were not considered to have failed. Freedom from PSA failure (FFPF) was calculated using the Kaplan-Meier method. Differences in failure rates were calculated using the log–rank test (26).

RESULTS

Overall, 19 patients experienced PSA failure. The actuarial 5-year FFPF rate was 86% (Fig. 1). Table 2 lists the 5-year FFPF rates analyzed according to the presenting disease characteristics. The only significant variable affecting PSA failure was the presenting Gleason score. The 5-year FFPF rate was 97% for a Gleason score of ≤6, 85% for a Gleason score of 7, and 76% for a Gleason score of 8–10 (p = 0.03; Fig. 2). A trend was noted for higher failure rates in SVB-positive patients, with a 5-year FFPF rate of 74% vs. 89% for all other patients (p = 0.06; Fig. 3). The initial PSA level and stage had no statistically significant effect on PSA failure. No statistically significant difference

Table 2. Effects of presenting disease characteristics on PSA failure rates

Factor	5-y Freedom from PSA failure (%)	*p*
PSA (ng/mL)		
≤10	87	
>10–20	89	
>20	79	0.4
Stage		
≤T2b	86	
≥T2c	85	0.95
Gleason score		
≤6	97	
7	85	
8–10	76	0.03
Seminal vesicle status		
Negative or not done	89	
Positive	74	0.06

Abbreviation: PSA = prostate-specific antigen.

was found in the 5-year FFPF rate between the 89 patients who had at least one high-risk feature (82%) and the 43 patients who had at least two intermediate-risk parameters (93%; $p = 0.09$).

To determine whether obtaining pathologic evidence of negative lymph node status conferred an advantage, an analysis of the effect of undergoing LPLND was performed. In general, patients undergoing LPLND tended to have slightly higher risk features than those not undergoing LPLND. No statistically significant difference was found in the 5-year FFPF rate between those undergoing LPLND (80%) and those not undergoing LPLND (90%; $p = 0.09$).

In addition, the effect of the EBRT dose was tested for its potential impact on FFPF. For the 98 patients treated with EBRT to ≤50.4 Gy, the 5-year FFPF rate was 86% vs. 85% for the 34 patients treated to EBRT doses >50.4 Gy ($p = 0.90$). No statistically significant differences were noted in FFPF with regard to D_{90} values for ^{103}Pd. The 5-year FFPF rate was 82% for patients receiving a ^{103}Pd D_{90} of <90 Gy ($n = 56$) and 87% for those receiving a ^{103}Pd D_{90} of ≥90 Gy ($n = 67$; $p = 0.48$). Four patients with ^{103}Pd implants did not have dosimetry analysis because of potential interference from hip prostheses.

All efforts were made to obtain posttreatment testosterone levels for all patients. Those patients who were followed outside of the treating institution often did not have testosterone levels available. In these patients, only follow-up PSA levels were available. Sixty-three patients, without evidence of biochemical failure, had testosterone levels drawn at least 1 year after HT. The last testosterone values were taken 17–100 months (median, 43 months) after treatment. The testosterone levels ranged from 20 to 939 ng/dL (median, 347 ng/dL). Of the 63 patients with available testosterone levels at least 1 year after HT completion, 82% had a level in the normal range (≥200 ng/dL), and only 5 patients had a level of <50 ng/dL.

The PSA levels measured at the last follow-up visit for those patients without PSA failure are presented in Fig. 4.

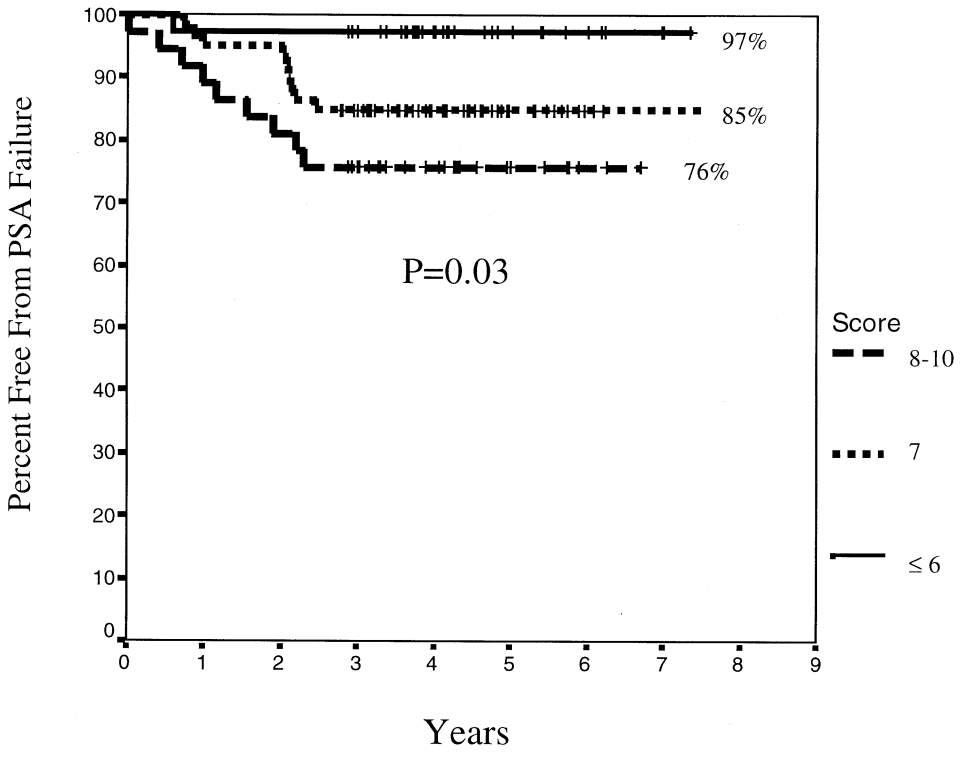

Fig. 2. Correlation of Gleason score and prostate-specific antigen (PSA) failure rate.

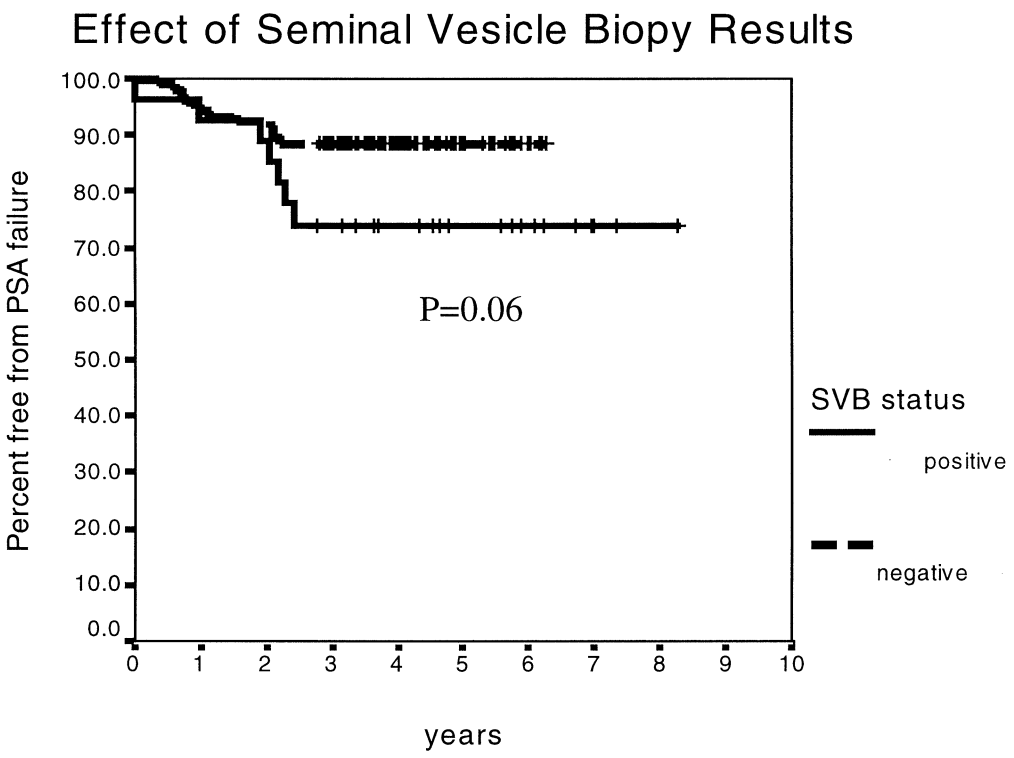

Fig. 3. Correlation of positive seminal vesicle biopsy (SVB) results and prostate-specific antigen (PSA) failure rate.

The vast majority (90%) had final PSA values of ≤0.2 ng/mL.

Forty-seven patients (36%) underwent posttreatment prostate biopsies 2 years after therapy. Nine (47%) of the 19 patients with PSA failure underwent biopsies, and 38 (33%) of 113 without PSA failure underwent biopsies. Forty-five patients (96%) had negative biopsies at 2 years. The 2 patents with positive biopsies did not have evidence of biochemical failure. These 2 patients underwent repeat biopsies 6 months later (at 2.5 years after EBRT completion),

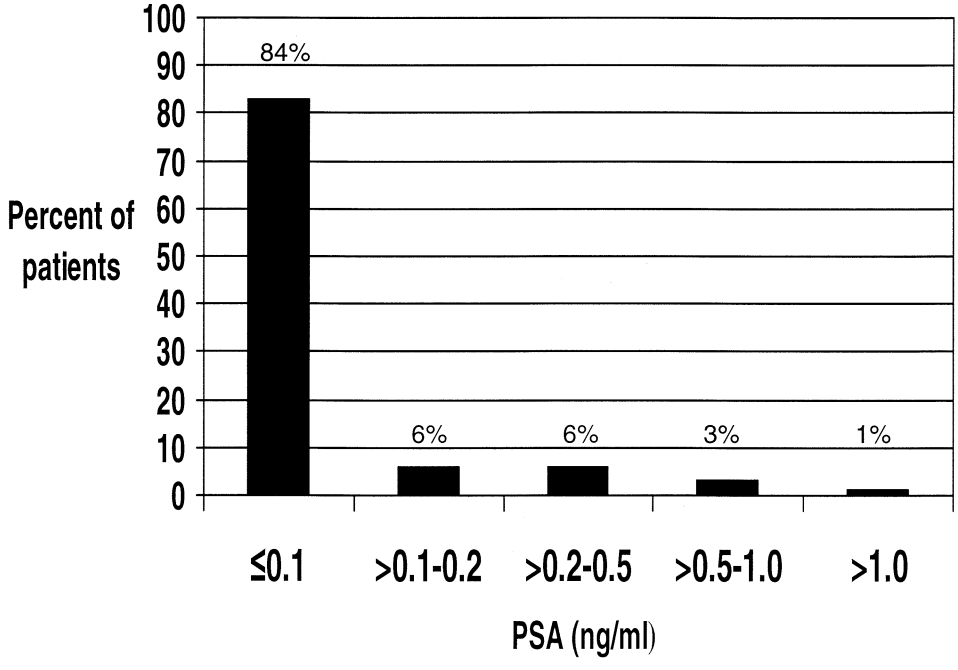

Fig. 4. Distribution of prostate-specific antigen (PSA) levels at last follow-up in patients free from biochemical failure.

and both were subsequently found to be negative. Therefore, 100% of patients had negative results at their last biopsy.

DISCUSSION

High-risk prostate cancer features have been associated with poor pathologic outcomes after radical prostatectomy (RP) (27). Increasing Gleason score, high PSA level, and advanced clinical stage have all been correlated with extracapsular disease extension, seminal vesicle invasion, and positive surgical margins. Tables and nomograms such as those developed by Partin *et al.* (28) have all demonstrated the increased incidence of the above-mentioned pathologic findings with higher risk features. These pathologic findings have also been associated with higher rates of biochemical failure (27, 29–34). One explanation for the association of these risk features with increased biochemical failure is that this cohort of patients has microscopic systemic disease at diagnosis. Although these patients are at increased risk of having metastatic disease, an alternative hypothesis to explain these failure rates is that RP and conventional RT may not provide optimal local control. By the nature of the anatomic constraints imposed on the surgery, it is often impossible to remove the prostate with adequate margins of normal tissue. RP in patients with a high risk of extracapsular extension and seminal vesicle involvement can result in tumor cells left behind in the surgical bed and hence inadequate local therapy (1, 6).

On the basis of this interpretation, it would seem that RT, which could provide better coverage of extracapsular disease, would fare better than RP in controlling high-risk disease. However, some of the initial data on PSA control after standard EBRT proved that this assumption was wrong, and that both methods yielded poor outcomes in high-risk prostate cancer. D'Amico *et al.* (1) found no statistically significant difference in the 5-year FFPF rate for EBRT (33%) compared with RP (15%) in high-risk patients. Other series have confirmed these suboptimal results after standard EBRT, with biochemical control rates ranging from 0% to 47% (2–5). Although, RT provides greater margins to control disease than RP, the above data demonstrate that standard doses of EBRT are inadequate to control the high volume of disease often found in high-risk patients.

To address the limitations of the above-mentioned conventional approaches to high-risk prostate cancer, in 1994, we developed a treatment regimen to enhance local control by focusing on two main innovations in RT: dose escalation and HT (24). The hypothesis was that the combination of neoadjuvant HT to reduce the tumor burden, concurrent HT and RT to enhance the cell kill, and radiation dose escalation would improve local control. Combined brachytherapy and EBRT was chosen as the best method to increase the intraprostatic dose and at the same time provide adequate radiation to the periprostatic tissue.

Evidence demonstrating improved biochemical control rates with the use of dose escalation and three-dimensional conformal EBRT has accumulated during the past decade (7, 11, 12, 16). High-risk patients have benefited the most from innovations in dose escalation. In addition, other methods of escalating the dose delivered to the prostate, such as combined brachytherapy and EBRT, have also shown promising results, as reported by a number of investigators (13–15, 35).

The beneficial effects of using HT with RT have been demonstrated in a number of retrospective and controlled trials. The randomized data from the European Organization for the Research and Treatment of Cancer and the Radiation Therapy Oncology Group all support improved outcomes with combined HT and EBRT vs. EBRT alone (9, 36–39). The 9-month duration of HT used in the current report was supported by data from Gleave *et al.* (8), in which 8 months of neoadjuvant HT was found to be superior to 3 months in terms of reducing positive margin rates and PSA nadir with RP.

The current approach is the first to use a standard regimen of 9 months of HT combined with brachytherapy and EBRT. This report is also unique in that it analyzed high-risk patients exclusively. Although it is difficult to compare the current series directly with other treatment regimens because of the variations in patient selection, the outcomes reported in this series compare very favorably with other treatment reports for high-risk patients. In a recent report of 547 high-risk prostate cancer patients treated with RP, the overall likelihood of being free from PSA failure at 5 years was 68%. In those patients with a Gleason score of 8–10, the actuarial freedom from failure rate at 5 years ranged from 19% to 47%, depending on the presenting PSA level (40). In the most recent Radiation Therapy Oncology Group study (9413), in which the optimal timing of HT and the RT technique was tested in high-risk patients, the best treatment arm produced a 4-year progression-free survival rate of 64% (41).

One potential criticism of the current series is that the results may have been more favorable owing to the surgical staging performed. The two minimally invasive procedures used in this study were SVB and LPLND. SVB has been shown to have a specificity approaching 100% and a sensitivity of 61–92% (42–44). However, the use of these procedures cannot be used to explain the superior outcomes compared with the RP series mentioned above, in which all patients had surgical staging (40). In addition, in the current report, the use of LPLND did not confer an advantage over patients who did not have the pelvic nodes surgically staged. Most EBRT reports do not routinely perform SVBs; thus, the current series probably contains a higher risk population of patients, because a positive SVB was an entrance criterion for the analysis. Another potential criticism is that 9 months of HT will result in a prolonged return of testosterone and an artificial delay in PSA recurrence. In the current study, testosterone levels returned to normal ($\geq$200 ng/dL) in 82% of the patients with available testosterone measurements at least 1 year after EBRT completion, with a median last level of 357 ng/dL. In addition, data that contradict the

assumption that a longer duration of HT will delay PSA recurrence were recently reported by Gleave *et al.* (45). Their study demonstrated that 8 months of neoadjuvant HT before RP did not decrease PSA recurrence compared with 3 months of HT, with respective recurrence rates of 50% and 40.8% ($p = 0.36$) (45).

Local control, as determined by digital rectal examination or prostate biopsy, in high-risk patients treated with RT or surgery alone has been poor in the past (1, 5, 6, 18, 46). Even as biochemical control rates have improved with novel radiotherapeutic approaches, positive posttreatment biopsy rates have remained relatively high. Pollack *et al.* (11) showed improved biochemical control with 78 Gy compared with 70 Gy ($p = 0.03$); however, the 8-Gy dose escalation did not significantly alter the biopsy results, with positive rates of 32% and 28%, respectively ($p = 0.52$). Despite showing biochemical and local dose-dependent responses with intensity-modulated RT, Zelefsky *et al.* (16) reported a 21% positive biopsy rate in intermediate- and high-risk patients treated with >75 Gy. Martinez *et al.* (17) showed a dose-dependent improvement in local control using combined EBRT and high-dose-rate brachytherapy. However, even in the high-dose group, the positive biopsy rate was 37% (17). The current approach of combining 9 months of HT with brachytherapy and EBRT resulted in negative biopsy outcomes of 100% at the last biopsy. Although biopsy was routinely recommended, patients with PSA failure were more likely to consent to biopsy than those free of recurrence. Forty-seven percent of patients with rising PSA values underwent biopsies vs. 33% of those free from biochemical failure.

CONCLUSION

A regimen of combined HT (9 months duration), brachytherapy, and EBRT produced excellent biochemical control rates in a group of high-risk prostate cancer patients. These results support the theory that enhanced local control (100% negative biopsy rate) can improve overall disease control in these patients.

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CLINICAL INVESTIGATION **Prostate**

DISEASE-SPECIFIC SURVIVAL FOLLOWING THE BRACHYTHERAPY MANAGEMENT OF PROSTATE CANCER

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Purpose: To determine disease-specific survival (DSS) and associated predictive factors after prostate brachytherapy.

Methods and Materials: A total of 1561 patients underwent brachytherapy for prostate cancer from 1990 to 2004 (median follow-up, 3.8 years). Treatment included brachytherapy alone ($n = 634$), brachytherapy and hormonal therapy ($n = 420$), and implant and external beam therapy ($n = 507$).

Results: The DSS and overall survival rates at 10 years were 96% and 74%, respectively. Gleason score significantly impacted DSS, with 10-year rates of 98%, 91%, and 92% for scores of ≤6, 7, and ≥8, respectively ($p < 0.0001$). Multivariate analysis revealed that PSA status after treatment had the most significant effect on DSS. Ten-year DSS rates were 100%, 52%, and 98%, respectively for patients without PSA failure ($n = 1430$), failure with a doubling time (DT) ≤10 months ($n = 64$), and failure with a DT >10 months ($n = 67$), respectively ($p < 0.0001$). In patients with PSA failure, DSS rates were 30%, 67%, and 98%, for those with DT ≤6 months, >6–10 months, and >10 months, respectively ($p < 0.0001$).

Conclusions: The 10-year DSS rate supports the efficacy of brachytherapy. Patients dying with disease within 10 years after treatment harbor inherently aggressive cancer with high Gleason scores and short DT.

Prostate cancer, Brachytherapy, Disease-specific survival, Doubling time.

INTRODUCTION

Over the last 15 years, investigators have focused on biochemical recurrence as a measure of treatment outcome. The use of prostate-specific antigen (PSA) has enabled clinicians to detect prostate cancer failure early, often years before clinical manifestations have appeared. A rising PSA level after definitive therapy might be due to local, nodal, or distant disease. The fact that different sources of a rising PSA level might lead to different PSA kinetics after therapy and the fact that prostate cancer has a long natural history mean that biochemical failure alone might not be the best endpoint for measuring treatment efficacy. Preventing death from cancer is the ultimate goal of prostate cancer therapy. More recently, investigators have begun reporting disease-specific survival (DSS) rates after therapy and have analyzed those factors that directly affect these rates. Because PSA failure is a precursor of eventual clinical relapse, the kinetics of PSA failure have also been examined to determine surrogate endpoints for disease-specific mortality (1). Recent analyses have focused on the PSA doubling time (DT) after radical prostatectomy and external beam radiation therapy and its impact on the development of metastatic disease and death from prostate cancer (2, 3). Most outcome reports after brachytherapy have focused on biochemical recurrence, but few have examined disease-specific mortality (4). The present report is the first brachytherapy series to examine long-term outcomes by specifically focusing on DSS. Disease-, treatment-, and posttreatment-related factors that could potentially affect DSS were examined to shed light on the natural history of the disease, as well the relationship between local control and prostate cancer death.

METHODS AND MATERIALS

Between 1990 and 2004, 1561 patients received treatment for localized prostate cancer with brachytherapy as part of the management. Patient age at time of treatment ranged from 41 to 88 years (median, 67 years). No patient had radiologic or pathologic evidence of metastatic disease. The clinical stages (1992 American Joint Committee on Cancer criteria), presenting Gleason scores, and presenting PSA values for the entire study population can be found in Table 1 (5).

Seminal vesicle biopsy was performed in 643 patients (41%). Indications for performing seminal vesicle biopsy were usually high-risk features: PSA level >10 ng/mL, Gleason score ≥7, or Stage ≥t2b. Overall, 51 of 643 patients (8%) were found to have adenocarcinoma invading the seminal vesicles. Laparoscopic pelvic lymph node dissections were performed in 226 patients

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Table 1. Presenting clinical characteristics

Characteristic	Number (%)
Stage	
T1a	3 (0.2)
T1b	9 (0.6)
T1c	697 (44)
T2a	327 (21)
T2b	345 (22.4)
T2c	148 (9.7)
T3a	25 (1.6)
T3b	3 (0.2)
T3c	3 (0.3)
Gleason score	
2–6	1073 (69)
7	330 (21)
8–10	158 (10)
PSA (ng/mL) 7.2 (0.1–300)	
≤10	1111 (71)
>10–20	312 (20)
>20	138 (9)

Abbreviation: PSA = prostate-specific antigen.
Values are *n* (%) or median (range).

(14%), and 7 patients had pathologically positive nodes. Details of these procedures have been previously described (6).

Patients were divided into risk groups on the basis of the presenting clinical characteristics. Low risk was defined as follows: PSA level ≤10 ng/mL, Gleason score ≤6, and Stage ≤t2a. Intermediate risk was defined as possessing only one of the following features: PSA level >10–20 ng/mL, Gleason score = 7, Stage = t2b. High risk included those with two or more intermediate-risk features or one or more of the following features: PSA level >20 ng/mL, Gleason score ≥8, Stage t2c–t3, or positive seminal vesicle biopsy.

Treatment

All patients were treated with brachytherapy with a real-time ultrasound-guided technique (7). Treatment regimens developed over time, so there was overlap in different risk groups being treated by different treatment regimens. Details of the development of these treatment schemas have been previously described (8). Treatments were divided into three main groups: brachytherapy alone (634 patients), brachytherapy and hormonal therapy (420 patients), and trimodality therapy (507 patients) with brachytherapy, hormonal therapy, and external beam irradiation.

Brachytherapy without external beam (with or without hormonal therapy) was performed with both ^{125}I (prescription dose, 160 Gy, Task Group 43 [TG43]) (858 patients) and ^{103}Pd (prescription dose, 124 Gy, National Institute of Standards and Technology [NIST] 1999) (200 patients). In general, ^{125}I was used for patients with Gleason scores of ≤6, and ^{103}Pd for those with scores ≥7. Most patients treated with brachytherapy alone were low-risk patients, although during the early years of the study period both intermediate- and high-risk patients received implant alone.

Hormonal therapy in conjunction with brachytherapy was used for two main reasons. The first use of hormonal therapy was for patients with large prostates (gland size >50 cm^3). It was given for 3 months before implantation and usually 2 to 3 months after implantation. The second use for hormonal therapy was as adjuvant therapy with brachytherapy for patients with intermediate- or high-risk features. In this case, the therapy was given for 3 months before and 3 months after implantation (9).

Trimodality therapy usually involved 3 months of hormonal therapy followed by a ^{103}Pd brachytherapy implant (prescription dose, 100 Gy, NIST 99) and 2 months later external beam radiation therapy to a dose of 45 Gy. The total duration of hormonal therapy was 9 months. In the earlier years of the study, lower implant doses were used with larger external beam doses. Details of this regimen have been previously described (10). Overall, when hormonal therapy was used it involved a luteinizing hormone-releasing hormone analog alone in 48% of patients and combined with an antiandrogen in 52%.

Dose equations

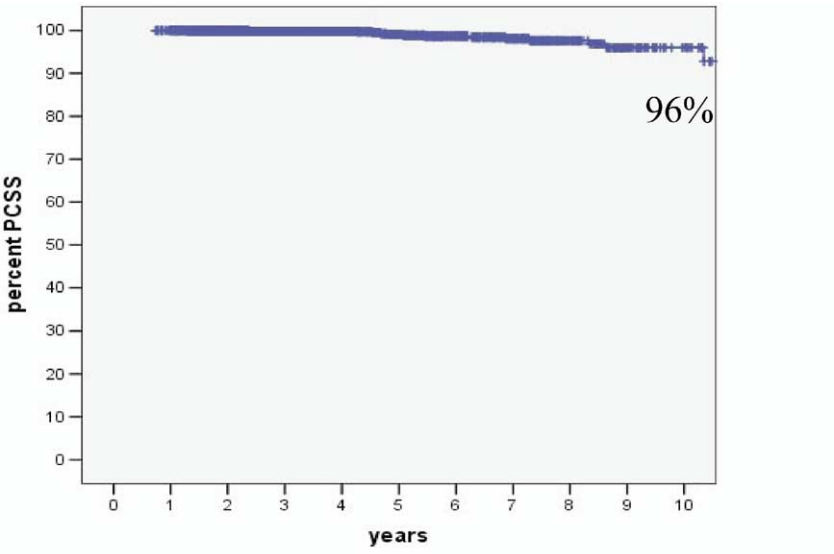
The dose delivered to the prostate was calculated with a 1-month postimplant CT-based dosimetric analysis. All patients were asked to return 1 month after implant for CT scanning. Dosimetry was performed in 1,501 patients. Reasons for not performing dosimetry were poor visualization due to hip prostheses or patient noncompliance. Implant dose was defined as the D90 (dose delivered to 90% of the gland from the dose–volume histogram) (11). To compare doses between different isotopes and between implant alone and combined implant and external beam, biologically effective dose (BED) equations were used. The BED values were obtained for both low-dose-rate permanent implants and the external beam portions of the treatment. An alpha/beta ratio of 2 was used in these equations. Details of these equations have been previously described (12–20). Patients treated with combined implant and external beam radiotherapy had their BED values for both treatments combined to determine the total BED. The BED values for the treatments ranged from 16 to 282 Gy_2 (median, 187 Gy_2).

Follow-up

All patients were asked to return for follow-up visits every 6 months after completion of treatment. Attempts to obtain follow-up information included the mailing of questionnaires. In addition, the final status of a patient was checked with the Social Security Death Index to determine the alive/dead status and date of death. All patients dying during the study period were followed up to determine cause of death and prostate cancer disease status. Follow-up was calculated from completion of treatment to last available follow-up date or date of death and ranged from 1 to 14 years (median, 3.8 years). Those patients with PSA failure (n = 131) were the ones at greatest risk of dying from prostate cancer, and they were followed from 1.2 to 14 years (median, 6.6 years).

Prostate-specific antigen failure was determined according to the American Society for Therapeutic Radiology and Oncology definition (21). In patients with a PSA failure, PSA DTs were calculated with first-order kinetics.

Posttreatment prostate biopsies were recommended in all patients. Overall, 450 patients underwent posttreatment biopsies. Biopsies (8–10 core samples) were recommended at 2 years after treatment. Repeat biopsies after this point were done for the following reasons: initial negative biopsy with continued rise in PSA levels or initial positive biopsy with no evidence of a rising PSA level. The outcomes for the biopsies were based on the last biopsy performed. Overall, 373 patients underwent one biopsy, 54 underwent two biopsies, 17 underwent three biopsies, and 6 underwent four or more biopsies. Biopsy results were read as positive



years	1	2	3	4	5	6	7	8	9	10
# @ risk	1541	1295	988	744	568	400	262	151	77	42

Fig. 1. Prostate cancer-specific survival (PCSS) for the entire study population.

or negative, with no indeterminate group (22). In general, patients with a rising PSA profile were more likely to consent to biopsy than those with stable PSA levels. Overall, 31 patients (7%) had a positive last biopsy. Those receiving biopsies were followed from 2 to 13.8 years (median, 6.5 years).

Patients were determined to have a death from prostate cancer if they died with the presence of metastatic prostate cancer. Neither the status of their last PSA measurement nor the therapies that they were receiving were used to determine death from prostate cancer.

Survival curves were determined with the methods of Kaplan and Meier. Differences in survival rates were calculated with the log–rank test. Multivariate analysis of survival was performed with Cox regression analysis. Differences in proportions were tested with the chi-square test (23).

RESULTS

The DSS and overall survival rates for the entire study population at 10 years were 96% and 74%, respectively (Figs. 1 and 2). For those patients dying of prostate cancer, the time from initial treatment to prostate cancer death ranged from 1.2 years to 10.3 years (median, 5 years). The time from PSA failure to prostate cancer death ranged from 2 months to 8 years (median, 3.8 years). Age at diagnosis had no effect on DSS. Patients aged ≤60, >60–70, and >70 years had DSS rates of 96%, 97%, and 94%, respectively ($p = 0.4$). The effect of the pretreatment disease factors, PSA level, Gleason score, clinical stage, and risk group on DSS can be seen in Table 2. In univariate analysis, Gleason score had the greatest effect on DSS ($p < 0.0001$). The effect of the treatment-related factors, treatment group, hormonal therapy use, and delivered dose can be seen in Table 3. Patients were divided into BED groups ≤100, >100–120, >120–140, >140–160, >160–180, and >180 Gy_2. There were no significant differences in DSS among the BED groups. The division of the population into two BED groups failed to show a significant dose–response relationship with DSS. Patients with BED ≤150 Gy_2 (171 patients) had a 10-year DSS rate of 98%, compared with a rate of 97% for those with BED values >150 Gy_2 (1,330 patients) ($p = 0.55$).

PSA status after therapy

The PSA status after therapy had a significant impact on DSS. Patients were divided into three groups: no PSA failure (1430 patients), PSA failure with a DT ≤10 months (64 patients), and PSA failure with a DT >10 months (67 patients). Ten-year DSS rates were 100%, 52%, and 98%, respectively ($p < 0.0001$) (see Fig. 3). The choice of a DT cut point of 10 months was based on a prior study of DSS after radical prostatectomy (3). Those patients experiencing a PSA failure were examined separately by using DT cut points of ≤6 months (43 patients), >6 months to 10 months (21 patients), and >10 months (67 patients). This analysis revealed 10-year DSS rates of 30%, 67%, and 98% ($p < 0.0001$), respectively (see Fig. 4).

In addition, the time to PSA failure was tested for its effect on DSS. On univariate analysis, patients failing ≤1 year after treatment (32 patients) had a worse 10-year DSS of 64%, compared with 88% for those failing >1 year after treatment (99 patients) ($p = 0.04$).

Posttreatment biopsy

Patients with a negative last biopsy ($n = 419$) had a 10-year DSS rate of 99%, compared with 83% for those with a positive last biopsy ($n = 31$) ($p = 0.007$). A multivariate analysis performed on those patients receiving posttreatment biopsies revealed that only DT significantly affected DSS ($p = 0.03$). Biopsy outcome, along with Gleason score, PSA level, risk

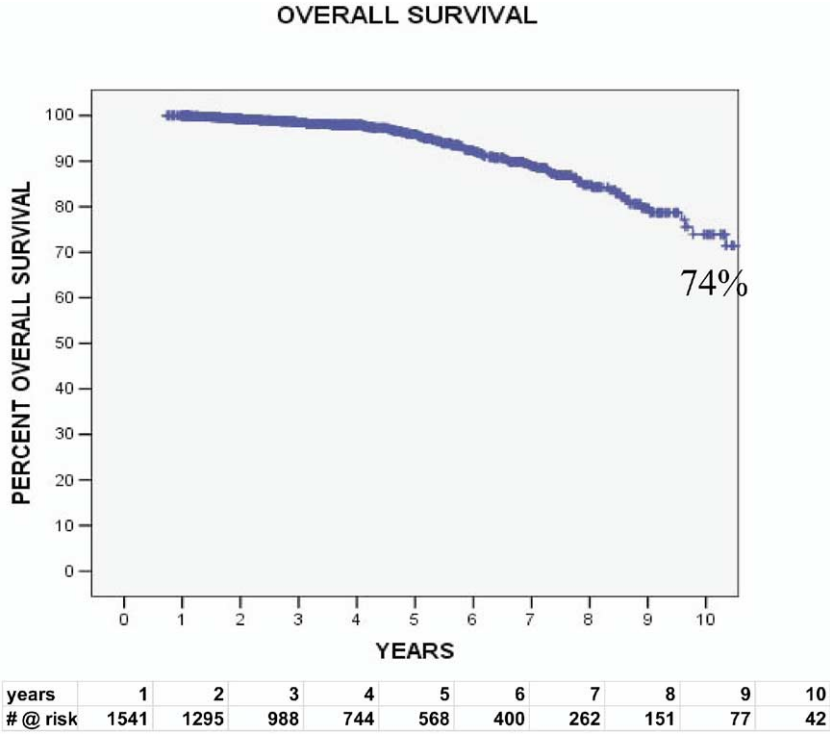


Fig. 2. Overall survival for the entire study population.

group, stage, and PSA failure time were not significant, with corresponding *p* values of 0.5, 0.8, 0.3, 0.9, and 0.9, respectively. Because patients dying of prostate cancer all experienced a PSA failure, the effect of biopsy in PSA failure patients was thought to be a more important issue to examine.

In PSA failure patients, those with negative biopsies (65 patients) had a 10-year DSS of 96%, compared with 77% for those with positive last biopsies (18 patients) ($p = 0.3$).

Multivariate analysis

A Cox regression analysis was performed to test the effect of disease and treatment-related factors on DSS (Table 4). This revealed that Gleason score was the only pretreatment/treatment factor to significantly affect DSS ($p = 0.003$). A multivariate analysis of these factors, as well as PSA status after treatment (no failure, failure with DT ≤10 months, failure with DT >10 months), revealed that the PSA status after therapy and Gleason score were the only significant factors to predict for prostate cancer death (Table 5). A similar analysis, done only on the 131

Table 2. Effect of disease-related factors on DSS

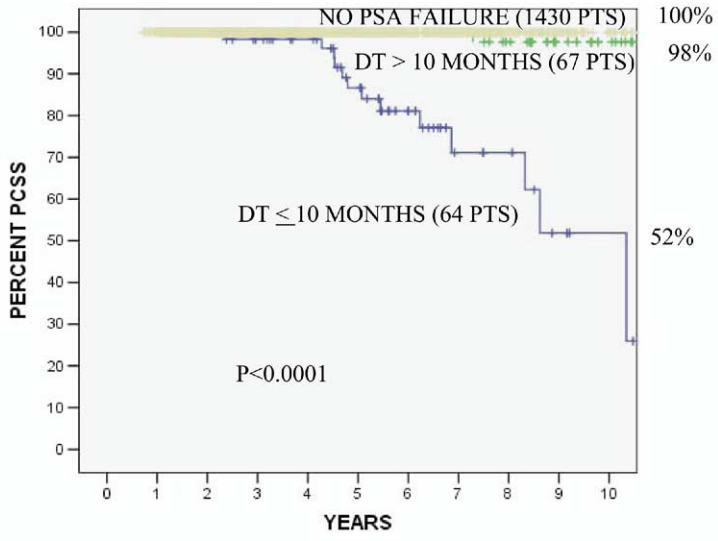
Factor	*n*	Deaths (*n*)	10-y DSS (%)
Gleason score			
≤6	1073	5	98
7	330	4	90.5
8–10	158	6	92 ($p < 0.0001$)
Clinical stage			
<t2a	1036	3	99
>t2b	345	6	95
≥t2c	180	6	88 ($p = 0.002$)
PSA (ng/mL)			
≤10	1111	6	99
>10–20	312	5	91
>20	138	4	93 ($p = 0.15$)
Risk group			
Low	680	1	99.6
Intermediate	360	2	98
High	521	12	92 ($p = 0.005$)

Abbreviation: DSS = disease-specific survival. Other abbreviation as in Table 1.

Table 3. Effect of treatment-related factors on DSS

Factor	*n*	Deaths (*n*)	10-y DSS (%)
Treatment group			
Implant alone	634	5	98
Implant + ADT	420	5	92
Trimodality	507	5	97 ($p = .05$)
ADT			
No	698	5	98
yes	863	10	92 ($p = 0.06$)
BED group (Gy_2)			
≤100	46	2	97
>100–120	33	0	100
>120–140	51	0	100
>140–160	101	3	92
>160–180	217	3	97
>180	1,053	4	99 ($p = 0.523$)

Abbreviations: ADT = androgen deprivation therapy; BED = biologically effective dose. Other abbreviation as in Table 2.



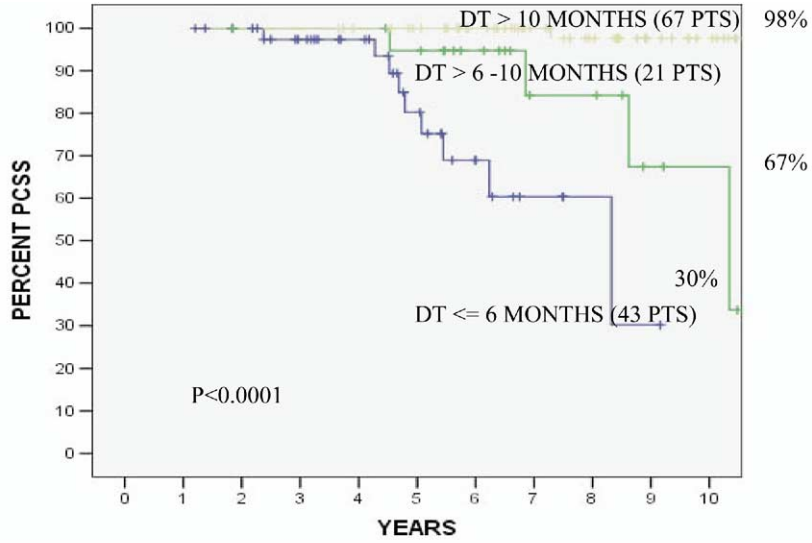
	YEARS	1	2	3	4	5	6	7	8	9	10
# @ RISK	NO FAIL	1410	1168	868	633	472	324	207	105	49	23
	DT>10M	66	66	66	64	61	54	44	37	28	17
	DT≤10M	63	60	53	47	35	22	11	9	4	2

Fig. 3. Effect of prostate-specific antigen (PSA) status after therapy on prostate cancer-specific survival (PCSS). DT = doubling time; M = months.

patients with a PSA failure, found that PSA DT was the only significant predictor of DSS (see Table 6).

DISCUSSION

Studies examining treatment outcomes after prostate brachytherapy have focused on PSA failure as an endpoint. These studies demonstrate that factors that are intimately related to local control are strongly correlated with biochemical control. Higher initial PSA values, greater percentage of positive biopsy cores, and greater clinical stage are all surrogates for increased local tumor burden, and have all been shown to significantly affect PSA failure (4, 24–26). In addition, implant dose has been found to be one of the most important predictors of biochemical control (11, 27, 28). These findings demonstrate the close relationship between local tumor eradication



	YEARS	1	2	3	4	5	6	7	8	9	10
NO@RISK	DT≤6M	43	40	33	27	17	9	4	2	1	0
	DT>6-10M	21	19	19	19	18	13	7	7	3	2
	DT>10M	67	67	67	64	61	56	44	37	24	17

Fig. 4. Effect of prostate-specific antigen doubling time (DT) on prostate cancer-specific survival (PCSS) in patients with PSA failure. M = months.

Table 4. Multivariate analysis of the impact of disease- and treatment-related factors on DSS

Factor	*p* value	Exp(B)	95% Confidence interval
Treatment group	0.85	0.84	0.15–4.7
ADT	0.54	2.03	0.20–20.4
Gleason score group	0.003	4.14	1.16–10.7
PSA groups	0.82	1.09	0.50–2.4
Stage group	0.14	2.04	0.78–5.3
Risk group	0.52	0.60	0.13–2.8
BED group	0.92	0.98	0.59–16

Abbreviations as in Tables 1, 2, and 3.

and PSA failure. Although biochemical control is an important endpoint, DSS might be more relevant in terms of measuring prostate cancer treatment outcome.

This study is the first following brachytherapy to specifically focus on DSS and the factors that affect it. It is important to examine those factors that can potentially affect DSS to shed light on the natural history of the disease and the relationship between local control and eventual death from the disease. In the current study, the overall DSS at 10 years was 96%, significantly higher than the 74% overall survival rate seen over the same period. This highlights the success of the therapy, the slow natural history of the disease, and the role that competing causes of mortality have in this study population (median age, 68 years). These results compare favorably with the 90.4% cause-specific mortality rate for the radical prostatectomy arm of the randomized trial of watchful waiting vs. surgical intervention reported by Bill-Axelson *et al.* (29). Our 96% DSS rate seems to be superior to the 85% cause-specific mortality rate seen in the watchful waiting arm of this trial, as well as to the results of other observational series (29, 30).

The major finding of this study is that those factors that reflect the biologic aggressiveness of the cancer have the greatest impact on DSS 10 years after therapy. In a multivariate analysis of both pretreatment and treatment factors, only the biopsy Gleason score significantly affected DSS. The effect of the posttreatment PSA profile on DSS also supports this conclusion. Prostate-specific antigen failure itself was not the major predictor of DSS. It was the kinetics of the rising PSA

Table 5. Multivariate analysis of factors predicting for DSS, including PSA status after treatment

Factor	*p* value	Exp(B)	95% Confidence interval
Treatment group	0.85	1.15	0.24–5.5
ADT	0.46	2.36	0.24–23.3
Gleason score group	0.01	3.39	1.3–8.7
PSA groups	0.67	0.85	0.39–1.8
Stage group	0.22	1.87	0.68–5.0
Risk group	0.47	0.58	0.13–2.5
BED group	0.94	1.01	0.62–1.7
PSA status	0.01	2.56	1.2–5.5

Abbreviations as in Tables 1, 2, and 3.

Table 6. Multivariate analysis of factors predicting for DSS in PSA failure patients

Factor	*p* value	Exp(B)	95% Confidence interval
Gleason score group	0.33	1.57	0.66–3.44
PSA groups	0.305	1.48	0.70–3.13
Stage group	0.872	1.15	0.21–6.20
Risk group	0.234	0.40	0.09–1.80
Doubling time	0.000	0.99	0.98–1.00
PSA failure time	0.70	1.0	0.99–1.00

Abbreviations as in Tables 1, 2, and 3.

profile that was most predictive. Patients who did not experience a PSA failure had 100% DSS, which was not different from the rate of 98% for those patients with a PSA failure and a DT >10 months. These rates were in marked contrast to the 52% rate seen in patients with a DT ≤10 months. The use of PSA DT as a surrogate for the biologic aggressiveness of the cancer has been demonstrated in both radical prostatectomy and external beam series (2, 3). The present study supports these findings. In fact, when the PSA status after therapy was factored into the multivariate analysis, it became, along with Gleason score, the most important predictors of DSS. Among those patients experiencing a PSA failure, there was an incremental worsening of DSS with shortening DTs. Patients with DTs >10 months, >6–10 months, and ≤6 months had 10-year DSS rates of 98%, 67%, and 30%, respectively. The PSA DT was the only factor that predicted for DSS among patients with a PSA failure in multivariate analysis.

Another interesting finding was that factors that have been closely associated with local control, such as PSA level and dose, did not predict for 10-year DSS. In prior studies, dose was the most significant predictor of both posttreatment biopsy outcome and PSA failure (11, 22). In the present study, dose (BED) showed no consistent affect on DSS. Among patients with a PSA failure, the results of the posttreatment biopsy did not affect DSS. A multivariate analysis performed on only patients receiving biopsies failed to demonstrate a significant effect of biopsy outcome on DSS. This suggests that those patients with the most biologically aggressive disease (those dying of prostate cancer within 10 years after treatment) probably harbor microscopic disseminated disease at onset. In these patients, local control would not be expected to have a significant impact of DSS. This has been demonstrated in the past in a study of retropubic ^{125}I prostate implants. In this study, local control had little impact on the development of metastatic disease in node-positive prostate cancer patients (31). During the first 10 years after therapy, the biologic aggressiveness of the tumor is the most important determinant of death from prostate cancer, and the surrogates for this aggressiveness are Gleason score before treatment and PSA kinetics after therapy. This is not to say that local control will have no effect on DSS, but longer follow-up might be needed to demonstrate this effect.

In conclusion, 10-year DSS after the brachytherapy management of prostate cancer supports the efficacy of this form of

cancer treatment. Patients dying of the disease within 10 years after treatment harbor inherently aggressive disease. Predictors of this type of biologic activity are Gleason score, pretreatment, and PSA kinetics after treatment. Within this time frame, predictors of local control have little impact on the risk of dying of disease.

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PHYSICS CONTRIBUTION

DOES PRIOR TRANSURETHRAL RESECTION OF PROSTATE COMPROMISE BRACHYTHERAPY QUALITY: A DOSIMETRIC ANALYSIS

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Purpose: To evaluate, in a retrospective review, prostate brachytherapy dosimetry outcomes relative to the transurethral resection of the prostate (TURP) cavity size to address the theoretical concern that an intraprostatic cavity may hinder adequate radioactive source placement.

Methods and Materials: A total of 73 patients who underwent prostate brachytherapy as part of their treatment of localized prostate cancer had a history of a prior TURP. Of these 73 patients, 37 underwent ^{125}I implantation, 19 ^{103}Pd implantation, and 17 partial ^{103}Pd implantation. The dose was calculated using the dose to 90% of the prostate gland (D_{90}) from the 1-month post-implant dosimetric analysis. The doses were normalized relative to 100% of the prescription dose. Archived transrectal ultrasound images were used to determine the maximal length and width of the visible residual TURP cavities. The prolate spheroid or symmetric egg shape was used to calculate each residual cavity volume. The derived volume of the TURP cavity was divided by the measured ultrasound volume of the prostate at brachytherapy, creating a percentage of volume measurement for each prostate. All p values, unless otherwise specified, were generated by comparing patients without a visible TURP defect with the subgroups of patients with a visible defect using the Student t test.

Results: A visible residual TURP defect was noted on the operative transrectal ultrasound images of 55 (75%) of the 73 patients with a history of TURP before brachytherapy. The 18 patients without a visible TURP defect had a median D_{90} of 96% and were used for subsequent statistical comparison. Thirty-six patients with a TURP defect <10% of the entire prostate volume had a median D_{90} of 109% ($p = 0.02$). Thirteen patients with a TURP defect between 10% and 20% of the prostate volume had a median D_{90} of 112% ($p = 0.03$). Six patients with a TURP defect >20% of the prostate volume had a D_{90} of 89% ($p = 0.43$).

Conclusion: A visible residual TURP cavity that is assumed to have a prolate spheroid shape and occupy ≥10% of a prostate volume did not appear to be a statistically significant hindrance to proper dosimetric outcome.

Brachytherapy, TURP, Dosimetry, Prostate cancer.

INTRODUCTION

Permanent prostate brachytherapy has become a common treatment option for localized prostate cancer. Initially, patient selection was limited to those with smaller prostates and no history of transurethral resection of the prostate (TURP) (1). As more experience has been gained, the initial strict selection criteria have been liberalized. Patients with a history of benign prostatic hyperplasia who had required surgical relief of their obstructive symptoms had previously been excluded from seed implantation. TURP results in resection of tissue occupying the bladder neck and a substantial proportion of the central and transition zones (2, 3). From the brachytherapist's perspective, the TURP cavity may create a technical hurdle, because the remaining central tissue may not be adequate to permit proper seed placement and, by extension, a suboptimal dose distribution may result.

In addition to the TURP cavity possibly creating a problem with internal needle and seed placement, the resected prostate gland often becomes asymmetric and irregular, making straight alignment of needles and sources difficult to ensure a homogeneous dose that encompasses the entire gland. To address these concerns, we reviewed the preoperative ultrasound-based images of the prostate gland in all our patients who reported a history of TURP before implantation and correlated the TURP cavity size with the relevant dosimetric parameters.

METHODS AND MATERIALS

Between January 1992 and December 2002, 73 consecutive patients with a history of prior TURP underwent implantation with the real-time technique and postoperative computed tomography (CT)-based dose evaluation.

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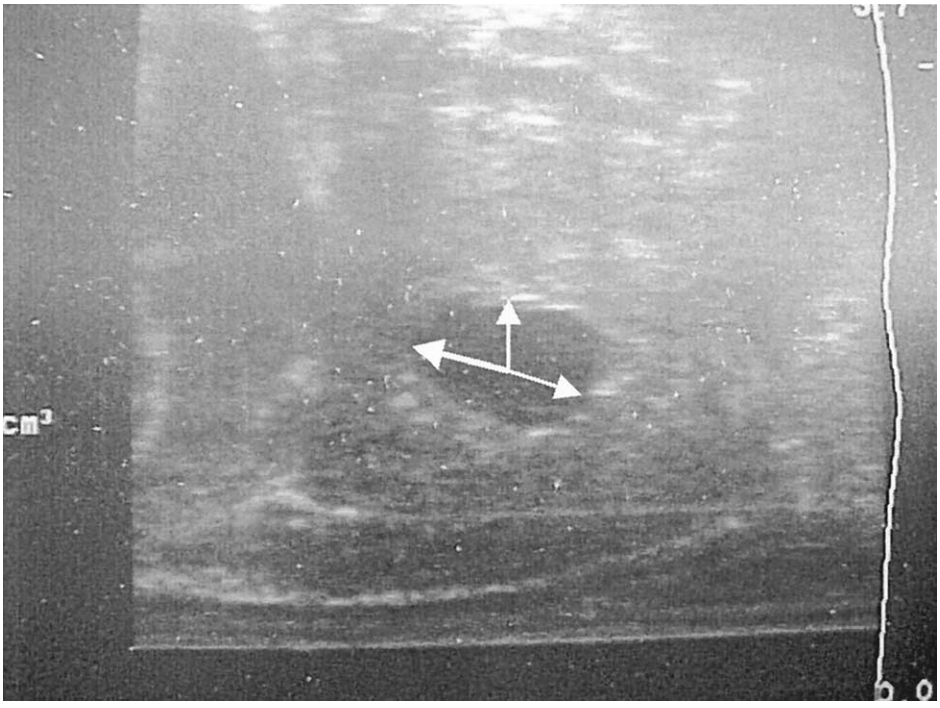

Fig. 1. Transrectal ultrasound image showing midline sagittal view of transurethral resection of the prostate cavity.

Technique

The implantation was performed using the previously described interactive ultrasound-directed technique (4, 5). The patient was placed in the extended dorsal lithotomy position, and a B&K model 8551 (1992–1995) or 8558 (1995 to present) biplanar ultrasound probe (B&K Medical, Wilmington, MA) was positioned in the rectum. A planimetry volume measurement with a 16F urethral catheter was performed using 5-mm transverse images of the prostate from the base to the apex. All transverse ultrasound images were printed and stored in the patient's chart. The volume was recorded, and the amount of activity to implant was determined by using an activity-per-volume table and, in later years, with an intraoperative computerized dosimetry system (Varian, Palo Alto, CA) (5, 6). The length of each prostate was determined from the mid-sagittal cut at the anterior, middle, and posterior aspects of the gland. The total number of seeds was determined by dividing the total activity indicated on a reference nomogram by the activity per seed to deliver a prescription dose of 160 Gy for ^{125}I, 115 Gy (before NIST-99) and 124 Gy (after NIST-99) for full ^{103}Pd and 90 Gy and 100 Gy, respectively, for partial ^{103}Pd implantation. A partial implant with ^{103}Pd uses a prescription dose of 100 Gy (American Brachytherapy Society recommendations), which is lower than the 124 Gy used for a full-dose implant. A partial implant implies that the dose from the implant is not the only dose delivered but is a component of the total dose (implant dose plus EBRT dose) (7). Generally, 75% of the total numbers of seeds required were implanted through the peripheral needles and 25% through the interior needles.

Determination of TURP cavity size

The ultrasound step-section images were reviewed to determine the size of the prostate TURP defect. A TURP defect was considered visible if it was $>$5 mm in diameter; this was necessary because all preimplant ultrasound measurements were done with a 16F urethral catheter, which has a diameter of approximately 5 mm. The catheter's position serves as a guide to intraoperative visualization and unavoidably alters the normal ultrasound image of the urethra as an arched-appearing closed slit (8). If the TURP defect was visible on transverse imaging, the midline sagittal and all additional transverse images were used to determine the maximal diameter and length using the ultrasound measurement grid and a ruler (Fig. 1). The residual cavity size was quantified by converting these two maximal measurements into a three-dimensional volume using the geometric formula for a prolate spheroid, $volume = (4/3)\pi hr^2$.

Postimplant CT-based dosimetry

Computed tomography-based dosimetry was performed 1 month after implantation with the ADAC Pinnacle system (ADAC Laboratories, Milpitas, CA) by taking 3-mm interval slices through the prostate volume. On every CT slice, the prostate, urethra, and rectum were contoured. All values were calculated with the American Association of Physicists in Medicine Task Group 43 (TG-43) formalism (9). Dose–volume histograms were generated of the prostate and urethra. For comparative analysis, the dose to 90% of the prostate gland (D_{90}) was converted to a percentage of the prescription dose, with 100% defined as the D_{90} of 160 Gy for full ^{125}I implantation, 124 Gy for full ^{103}Pd implanta-

Table 1. Diameter, saggital length, and volume measurement of 55 TURP cavities visualized using ultrasound images

Isotope	Patients (*n*)	Resected cavity diameter (cm)	Urethral cavity length (cm)	TURP cavity volume (cm^3)
Full ^{125}I	30	1.1 (0.5–1.5)	1.7 (0.5–4)	2.6 (0.3–9.4)
Full ^{103}Pd	15	1.3 (0.5–2.5)	1.4 (0.5–2.5)	3.4 (0.5–16.4)
Partial ^{103}Pd	10	1 (0.8–1.2)	2 (0.5–3)	2.1 (0.3–4.5)

Abbreviation: TURP = transurethral resection of prostate.
Data in parentheses are ranges.

tion, and 100 Gy for partial ^{103}Pd implantation. The changes instituted in the 1990s by issuance of the TG-43 and the NIST-99 recommendations were accounted for by normalizing the prescription doses to the prostate implants over time (9, 10).

Statistical analysis

The percentage of the volume of the TURP cavity relative to the entire measured volume of the prostate provided the basis of dosimetric analysis. The 73 patients were divided into four groups: 18 patients without a visible defect, 36 patients with a defect that was <10% of the entire volume of the prostate, 13 patients whose defect was between 10% and 20%, and 6 patients with a defect >20% of the prostate volume. In addition, a match-paired analysis was done comparing the D_{90} values of the first 10 implants, without prior TURP (taken from a database of 1980 patients implanted between January 1992 and December 2002), yearly with the D_{90} outcomes of the patients implanted with a prior history of TURP. All *p* values, unless otherwise specified, were generated by comparing patients without a visible TURP defect with the subgroups of patients with a visible defect using the Student *t* test.

RESULTS

Of the 73 patients with a prior history of TURP, 37 were treated with a full ^{125}I implant, 19 with a full ^{103}Pd implant, and 17 with a partial ^{103}Pd implant. The mean prostate volume measured with planimetry before implant was 31.1 cm^3 (range, 8.4–85 cm^3); the mean prostate volume was 33.7 cm^3 (range, 8.8–89 cm^3) 1 month after implantation by CT-based dosimetry. The mean pretreatment ultrasound prostate volume was 33.1 cm^3 for ^{125}I, 25.7 cm^3 for ^{103}Pd, and 32.4 cm^3 for partial ^{103}Pd. The mean D_{90} for patients who were treated with ^{125}I, ^{103}Pd, and partial ^{103}Pd was 109% (95% CI, 104.3–113.7%), 98% (95% CI, 85.4–110.6%), and 104% (95% CI, 95–113%), respectively. The mean D_{90} of all patients was 105% (95% CI, 100.6–109.5).

Of the 73 patients, 55 (75.3%) had a visible TURP defect. A defect was visible in 30 (81%) of the 37 patients who underwent ^{125}I implantation, 15 (79%) of the 19 patients treated with full ^{103}Pd implantation, and 10 (58.8%) of the 17 patients treated with partial ^{103}Pd implantation. The mean TURP defect diameter for all patients was 1.2 cm (range, 0.5–2.5 cm), and the mean urethral length defect was 1.7 cm (range, 0.5–4 cm; Table 1). The mean TURP volume for all implants was 2.7 cm^3 (range, 0.3–16.4 ^{3}). The TURP cavity volume measurement was converted to a percentage of the measured prostate volume values, revealing a mean TURP cavity size for all visible defects of 10% (range, 1–68%) of the measured volume of the prostate. The mean TURP cavity volume was 8.3% for ^{125}I, 14.6% for full ^{103}Pd, and 8.2% for a partial ^{103}Pd implant. Overall, 24.7% had no defect, 49% had a defect of <10%, 17.8% had a defect between 10% and <20%, and 8.2% had a TURP defect >20% of the prostate size (Table 2).

Of the entire group of 73 patients, the 18 patients (24.7%) without a visible TURP defect had a median D_{90} of 96% (range, 36–127%) of the prescription dose. Of the 73 patients, 36 (49%) had a TURP defect that was <10% of the entire prostate volume and had a median D_{90} of 109% (range, 62–143%; $p = 0.02$). For the 13 patients (17.8%) with a TURP defect between 10% and <20% of the prostate volume, the median D_{90} was 112% (range, 88–138%) of the prescription dose, significantly greater than for patients who did not have a visible TURP defect ($p = 0.03$). Six patients (8.2%) had a relatively large TURP defect of >20% of the prostate volume. Their

Table 2. Mean values of dosimetric parameters of patients divided by isotope

Isotope	TURP volume as percentage of prostate volume	Ultrasound volume (cm^3)	Prescription dose (%)	V_{150} (%)
Full ^{125}I	8.3	2.6	109	59
Full ^{103}Pd	14.6	3.4	98	69
Partial ^{103}Pd	8.2	2.1	104	57

Abbreviations: TURP = transurethral resection of prostate; V_{150} = volume of prostate receiving 150% of prescription dose.

Table 3. Dosimetric parameters of patients partitioned by TURP defect size

TURP volume as percentage of prostate volume	Prescription dose (%)	V_{150} (%)	U_{30}*		
			^{125}I	^{103}Pd	Partial ^{103}Pd
None	96	55	149	126	122
<10	109	65	149	151	122
10–19	112	62	136	147	128
≥20	89	51	189	149	146

Abbreviations: U_{30} = dose received by 30% of urethral volume; other abbreviations as in Table 2.
* All urethral doses are reported as percentage of the prescription dose.

median D_{90} was 89% (range, 74–104%) of the prescription dose compared with the D_{90} of 96% for patients without a visible TURP ($p = 0.43$; Table 3). No statistically significant relationship was found between the volume of the prostate receiving 150% of the prescription dose and the TURP size. In addition, because of the heterogeneity of the implant isotope and treatment strategy, it was not possible to compare meaningfully the urethral dose, as quantified by the greatest dose received by 30% of the urethral volume, among the patients with and without a TURP defect.

Of the 6 patients with a TURP defect of >20% of the prostate volume, 3 (50%) had a D_{90} of <90% of the prescription dose in contrast to 4 (8%) of the 49 patients with a visible defect of <20% of the prostate volume ($p = 0.02$, Pearson's chi-square test). Of the 3 patients with a large TURP defect (>20%) and a D_{90} of <90% of the prescription dose, 2 had undergone implantation in 1994. A matched-pair analysis of the D_{90} outcomes of the first 10 patients implanted in 1994 revealed a mean D_{90} of 78.9% in patients without a history of prior TURP vs. 90.2% in the 7 patients with a history of TURP ($p = 0.26$). Table 4 compares the yearly D_{90} values of all patients with a history of prior TURP relative to the dosimetry outcomes of the first 10 patients implanted in each year from 1992 to 2002. The findings reveal that in each year no statistically significant difference resulted between the two treatment groups.

Eight patients with a history of multiple TURP procedures had a mean cavity volume of 3.7 cm^3 (95% CI 1.54–5.86) vs. 2.5 cm^3 (95% CI, 1.85–3.15) for patients with a single procedure ($p = 0.13$). In addition, patients who had undergone TURP <5 years before implantation had larger TURP cavities, with a mean of 3.3 cm^3 (95% CI, 2.14–4.45) vs. 1.6 cm^3 (95% CI, 1.25–1.95 cm^3, $p = 0.03$) if TURP had been performed >5 years before implantation.

Forty-five patients had undergone hormonal therapy for 6–9 months (3 months before implantation and 3–6 months after). The mean size of the hormone-treated prostates was 28 cm^3 (range, 8.5–77.5 cm^3) vs. 35.9 cm^3 (range, 9.2–85 cm^3) for the untreated ones ($p = 0.03$). No statistically significant difference was found in TURP cavity size as a percentage of the measured prostate volume between the hormone-treated patients (9.6%; 95% CI, 7.6–11.6%) and the untreated patients (10.6%; 95% CI, 5.6–15.6%; $p = 0.72$). In addition, the dosimetry results were similar for the two groups, with a mean D_{90} of 107% (95% CI, 101–113%) of the prescription dose for the treated patients vs. 106% (95% CI, 97–115%) for the untreated patients ($p = 0.84$).

When evaluated according to prostate volume, 43 pa-

Table 4. D_{90} as percentage of prescription dose for first 10 implants of each year from 1992 to 2002 vs. TURP patients implanted in that year

Year	Mean D_{90} (%) first 10 implants each year	History of TURP		
		Implants with history of TURP (*n*)	Mean D_{90} (%) history of TURP	*p*
1992	58.4	1	35.9	—
1993	67.3	1	127.1	—
1994	78.9	7	90.2	0.26
1995	91.9	10	100.8	0.32
1996	94.2	11	111.3	0.06
1997	110.5	13	110.8	0.95
1998	110.1	3	109.9	0.99
1999	111.8	4	115.6	0.99
2000	113.6	1	102.8	0.99
2001	116.4	17	107.2	0.07
2002	109.3	5	114.5	0.41

Abbreviations: D_{90} = dose to 90% of prostate gland; TURP = transurethral resection of prostate.

Table 5. Number of inner and outer seeds and needles partitioned by TURP cavity size

TURP volume as percentage of prostate volume (%)	Inner seeds (*n*)	Outer seeds (*n*)	Inner/total seeds ratio (%)	*p*	Inner needles (*n*)	Outer needles (*n*)	Inner/total needles ratio (%)	*p*
None	21	62	26	—	6	14	40	—
≤10	23	65	26	0.98	7	14	47	0.34
11 to ≤20	26	67	26	0.71	7	14	44	0.68
>20	23	56	24	0.73	4	13	22	0.07

Abbreviation: TURP = transurethral resection of prostate.

tients had a preimplant volume of <30 cm^3 and 30 patients had a volume from 32 to 85 cm^3. Patients with a prostate volume of <30 cm^3 had a mean D_{90} of 102% (95% CI, 96–108%), and patients with a volume of >30 cm^3 had a mean D_{90} of 109% (95% CI, 102.6–115.4%, $p = 0.14$). In addition, patients with a prostate size >30 cm^3 had a mean TURP cavity volume of 3.3 cm^3 (95% CI, 1.8–4.8); smaller prostates were noted to have a mean TURP volume of 2.3 cm^3 (95% CI, 1.7–2.9, $p = 0.17$). A trend was noted toward larger relative TURP cavity sizes among prostates <30 cm^3, with a mean percentage volume of 12% (95% CI, 8–16%) compared with larger prostates with a relative TURP cavity size of 7% (95% CI, 4.5–9.5%, $p = 0.07$).

Table 5 outlines the comparison of the number of needles and seeds used in each implant by the relative size of the TURP cavity. By comparing the ratio of the number of inner needles and seeds used in each patient with the total number of needles and seeds used, one can test the influence of prostate TURP defect size on needle and seed placement while controlling for variations in prostate size. A trend was noted among patients with the largest TURP defect toward a more peripherally loaded implant. The ratio of inner to total needles placed was 22% (95% CI, 2–42%) for patients with a visible TURP; the difference in the ratio of inner to total needles used among patients without a visible TURP defect (40%, 95% CI, 30–50%) was not statistically significant ($p = 0.07$). No statistically significant associations were noted in the ratio of inner to outer seeds used in the implant.

DISCUSSION

Prior TURP has historically been considered a relative contraindication for performing permanent seed brachytherapy (1). This concern arose from both the reported high complication rate in patients with a history of TURP and the technical difficulty in achieving adequate dose after TURP (11). Brachytherapy techniques that use a more peripheral approach to implantation have not been shown to be associated with increased morbidity in this setting (12).

This is the first study to examine the effect of prior TURP on the dosimetry outcome by examining the size of the TURP cavity. As demonstrated in this series, the TURP cavity is both visible and measurable by ultrasonography, revealing a relatively diverse anatomic variation among patients with a history of prior transurethral surgery. This variation did not appear to influence the quality of peripherally loaded implants in terms of the D_{90}, even though 26% of the patients were missing >10% of the central prostate volume. Also, no statistically significant influence was found on the volume of the prostate receiving 150% of the prescription dose; we were unable to directly compare the doses to 30% of the urethral volume because the variety in isotope and treatment strategies did not provide sufficient numbers for analysis.

We detected an influence on our implant technique of the largest TURP cavities, with a trend toward using relatively fewer centrally placed needles in those patients compared with in patients without an identifiable TURP defect. We believe the main reason for this was our emphasis on the interactive ultrasound-directed peripheral loading technique, which allows the central portion of the gland to remain free of very-high-dose regions. In addition, the real-time nature of our implants allows for intraoperative improvisation when such variations in expected anatomy as a significant TURP defect are identified. When reviewing the postimplant CT studies, the TURP defects were noted only among 18 (25%) of the 73 patients rather than the 55 (75%) detected by reviewing the ultrasound studies. The implication is that preplanning strategies reliant on CT volumetric studies may not be adequate to detect variations in the prostate's expected anatomy compared with the intraoperative ultrasound studies. Therefore, CT-based preplanning, although adequate for a large proportion of individuals with the expected anatomy, may fail to discern the subtleties in the anatomy of the central portion of the prostate (12).

Our series also offers some insight into the natural history of a TURP cavity. The patients who underwent TURP several years before implantation had smaller residual cavities than those who had undergone TURP more recently. The greater the interval between TURP and implantation, the more likely the prostate will grow and "fill in" this defect. This finding was dramatic in terms of the patient with the largest residual cavity of 68% of the TURP defect volume, in that his TURP had been approximately 2 months before the brachytherapy procedure. This observation may

be clinically useful in terms of patient selection if investigators are able to correlate the anatomic variation of the TURP cavity with the observed range of adverse clinical outcomes previously reported among patients with a prior history of TURP and prostate radiotherapy (13–15).

The use of hormonal therapy in this patient population might be a concern because the reduction in prostate size could inadvertently increase the proportion of the gland occupied by the TURP cavity. Our data suggest that treatment with hormone suppression therapy did not appear to affect the relative size of the residual TURP cavity or the resulting dosimetry by preferentially shrinking the peripheral zone over the central zone. Androgen deprivation, although reducing the prostate size, does not proportionally increase or decrease the TURP defect. Thus, it should be safe to use neoadjuvant hormonal therapy for gland shrinkage or for high-risk patients with a visible TURP cavity on ultrasonography (16).

CONCLUSION

This is the first study to evaluate intraprostatic anatomic variation in patients with a history of TURP and to correlate it with the postimplant dosimetric parameters. Patients with substantial TURP defects received the same quality of implantation as patients with no, or a small, TURP defect. Additional investigation on the relationship between cavity size and long-term morbidity is warranted to delineate whether this variable affects the late emergence of urinary incontinence.

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Effect of low dose-rate prostate brachytherapy on the sexual health of men with optimal sexual function before treatment: analysis at ≥7 years of follow-up

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OBJECTIVE

To evaluate the effect of low-dose rate prostate brachytherapy on the sexual health of men with ≥7 years of prospective evaluation and optimum sexual function before treatment.

PATIENTS AND METHODS

In all, 223 patients with T1b to T3a prostate cancer and a median (range) age of 66 (50–82) years were treated with permanent seed implantation from November 1990 to March 1998. They were followed for a median (range) of 8.2 (7–14.1) years using prospective quality-of-life measures. Erectile function (EF) was assessed using a physician-assigned score and beginning in June 2000; the validated International Index of EF (IIEF-5) was used as a complementary method to quantify late EF. No adjustment was made to differentiate sexual function with or with no pharmacological intervention for EF. Pearson's chi-square test and Student's *t*-test were used to compare the groups.

RESULTS

Of the 223 men, 131 (59%) had optimal EF before their brachytherapy; of these, 51 (40%) at the last follow-up evaluation were using either a phosphodiesterase type 5 inhibitor (44, 86%), yohimbine (two, 4%) or alprostadil (five, 10%). The age at implantation was highly predictive of current EF; 23 of 25 (92%) men aged 50–59 years had a current EF of ≥2; those aged 60–69 and 70–78 years had an EF of ≥2 in 48/75 (64%) and 18/31 (58%) ($P = 0.01$). A current IIEF-5 score of ≥16 also correlated highly with age at implant, i.e. 50–59, 16/25 (64%); 60–69, 20/75 (27%) and 70–78 years, 6/31 (19%) ($P < 0.001$).

CONCLUSION

Patients aged <60 years and with optimal EF before low-dose rate prostate brachytherapy have a very high probability of long-term EF.

KEYWORDS

prostate brachytherapy, prostate cancer, erectile dysfunction, IIEF-5

INTRODUCTION

Modern techniques for treating localized prostate cancer, including radical prostatectomy (RP), external beam radiotherapy (EBRT) and brachytherapy, have similar cancer-specific survival rates [1–3]. Treatment decisions for these patients are often difficult because of a lack of long-term toxicity data. All three treatments might result in the development of erectile dysfunction (ED), which occurs in up to 75% of patients [4–6]. Permanent ED is especially troublesome for younger and more sexually motivated men. While there are no randomized trials addressing this issue, a recent meta-analysis of non-randomized data summarized the effects of prostate cancer treatment on erectile function (EF) in 54 published articles [7]. The rate of ED after standard RP, a nerve-sparing RP, EBRT, EBRT plus brachytherapy and brachytherapy alone were 75%, 66%, 45%, 40% and 24%, respectively [7]. In addition, it is well established that the rates of ED after surgery, EBRT or brachytherapy increase with time [8,9]. Therefore, this report focuses on 131 patients with optimal EF before prostate brachytherapy who were followed for ≥7 years.

It is likely that the development of ED after prostate brachytherapy is multifactorial. Possible patient- and therapy-related factors include sexual function before treatment, age, medical comorbidities, genetic predisposition, method of data collection (patient-reported vs physician-reported), length of follow-up, dose to erectile tissues, use of hormonal therapy and use of erectile aids [10,11]. While our previous studies focused on technical and genetic predictors of brachytherapy-induced ED, the primary goal of the present study was to identify the patient-reported factors associated with late sexual dysfunction.

PATIENTS AND METHODS

Between June 1990 and March 1998, 586 men had prostate brachytherapy at Mount Sinai Hospital; the EF was followed prospectively for ≥7 years in 223 (38%) of these men, but in the remaining 363 with <7 years of follow-up for ED the many attempts to acquire the information were unsuccessful. Our practice pattern is to offer all patients a long-term prospective evaluation with several quality-of-life measures, and therefore the 223 men in the present report had chosen to continue their follow-up with the radiation oncology department rather than, or along with, their urologist.

All patients had biopsy-confirmed adenocarcinoma with the pathology reviewed at the Mount Sinai Medical Center. Patients were staged according to the 1992 American Joint Cancer Commission standard [12].

TABLE 1 The characteristics of the 223 men with or without ≥7 years of follow-up

Characteristic	≥7 years (223)	<7 years (363)	P
edian (range) age, years	66 (50–82)	68 (50–86)	0.02*
T stage, n (%)			
Recurrent	0	7 (2)	
T1a	1 (0.5)	0	
T1b	13 (6)	2 (<1)	
T1c	61 (27)	106 (29)	
T2a	44 (20)	106 (29)	
T2b	70 (31)	83 (23)	
T2c	27 (12)	53 (15)	
T3a	7 (3)	4 (1)	
T3b	0	1 (<1)	
T3c	0	1 (<1)	
Median (range) PSA, ng/mL	8.5 (1.2–300)	8 (1–120)	0.24*
≤10	133 (60)	236 (65)	0.19*
>10	90 (40)	127 (35)	
Median (range) Gleason score	6 (2–9)	6 (2–9)	
2–6	172 (77)	250 (69)	0.03*
7–9	51 (23)	113 (31)	
Baseline EF score 3 (normal)	131 (59)	179 (49)	0.02†

**Student's t-test; †Pearson's chi-square test.*

TABLE 2 The treatment characteristics of 223 patients with ≥7 years of follow-up and those of the subset of 131 patients with optimal EF within the group

Characteristic	All	Optimal EF
N (%)		
Isotope:		
^{125}I full-implant	127 (57)	79 (60)
^{103}Pd full-implant	73 (33)	43 (33)
partial-implant	23 (10)	9 (7)
Median (range) D90, Gy		
^{125}I full-implant	159.5 (35.5–256.3)	161.9 (35.5–256.3
≤140	38 (30)	19 (24)
>140	89 (70)	60 (76)
Hormones	23 (18)	16 (20)
^{103}Pd-103, full-implant	108.8 (35.0–153.9)	105.2 (42.6–153.9)
≤124	55 (75)	32 (74)
>124	18 (25)	11 (26)
Hormones	58 (80)	37 (86)
^{103}Pd partial-implant	80.7 (28.0–148.9)	80.3 (56.6–78.9)
≤100	16 (70)	7/9
>100	7 (30)	2/9
Hormones	23 (100)	9/9
EBRT dose, Gy		
45	2 (9)	1/9
48.6	1 (5)	1/9
59.4	20 (87)	7/9

Patient and tumour characteristics are outlined in Table 1. Brachytherapy was administered via the real-time transperineal approach using TRUS to direct the placement of each radioactive source within the prostate [13]. The implant characteristics are shown in Table 2. The prescription dose for ^{125}I-implants was 160 Gy, corrected for the TG-43 recommendation [14]. The prescription dose of ^{103}Pd-implants was 124 Gy for a full implant and 100 Gy for partial implants, following the National Institute of Standards and Technology 1999 recommendations [15]. Patients treated with partial implants received supplemental EBRT of 45 Gy to 59.4 Gy [16]. Patients returned at ≈4 weeks after the implant for detailed CT-based dosimetric analysis; EBRT was begun 8 weeks after the implantation. The follow-up included a DRE and serial PSA measurements. Biochemical failure was defined using the American Society for Therapeutic Radiation and Oncology consensus definition [17]. To accurately assess ED after brachytherapy, for the entire group, patients treated with salvage hormone therapy were included in the study.

All patients had a detailed history taken and a physical examination before implantation, followed by a directed history and physical examination at 6-month intervals afterward. ED was assessed using the Mount Sinai EF (MSEF) physician-assigned scoring system, i.e. 0, complete inability to have erections; 1, able to have erections but insufficient for intercourse; 2, can have erections sufficient for intercourse but considered suboptimal; and 3, optimal EF. The derivation and relevance of this scoring system were described previously [18,19]; a score of 0 or 1 was considered as ED. Beginning in June 2000, the validated International Index of Erectile Function (IIEF-5) was used as a complementary method to better quantify late ED [20], with a score of ≥16 on the IIEF-5 defining adequate EF; a score of 16 was found to result in good EF in a group of 124 men given sildenafil in a randomized clinical trial of men who had a baseline mean IIEF-5 score of 7.7 [21]. In addition, investigators from the Cleveland Clinic found that a score of ≥16 on the IIEF-5, using the 'medicated urethral system for erection' after RP, predicted continued sexual activity, whereas a lower score predicted the discontinuation of erectile attempts using this treatment [22]. Because of the relatively recent use of the IIEF-5, the present analysis did not allow a

prospective evaluation in the present patients and the last completed form was used for the study. All patients included in the study were entered based on guidelines approved by the Mount Sinai Medical School institutional review board.

The results were analysed using standard statistical software, with differences in proportions tested using the chi-square statistic, and difference in means with Student's *t*-test, with a two-sided $P \leq 0.05$ considered to indicate statistical significance in all tests.

RESULTS

The median (range) follow-up of the 223 patient was 8 (7–14) years; those with a longer follow-up appeared to be in a more favourable prognostic category, with a statistically lower Gleason sum of 2–6 in 77% ($P = 0.03$). There was a trend to better baseline EF at implantation among patients with ≥7 years of follow-up, with 131 of 223 (58.7%) having normal EF, vs 179 of 363 (49%) ($P = 0.02$; Table 1). The incidence of diabetes, hypertension, smoking and use of adjuvant hormone therapy, distribution of isotopes used for treatment, and EBRT dose were evenly distributed between both the patients followed for ≥7 years and those lost to follow-up and not assessed for EF.

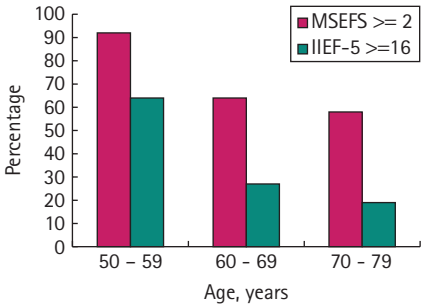
Of the 131 patients with an optimal MSEF score (of ≥3), 42 (32%) developed ED; the mean age at implantation of these men was 67 (57–78) years, vs 63 (50–78) years ($P < 0.001$) for those who maintained EF. Patients who were 50–59 years old when implanted had an potency rate of 92%, based on an MSEF score of ≥2, or 64% for an IIEF-5 of ≥16, at ≥7 years of follow-up; those aged 60–69 years had a 64% potency rate by MSEF score and 27% by the IIEF-5. Relatively elderly patients, implanted when aged 70–79 years, all of whom are now >76 years old, had a MSEF score of ≥2 in 58% and a IIEF-5 of ≥16 in 19% (Fig. 1). There was no difference between the development of ED based on the isotope used. Of the 131 men with normal EF before implantation, 60% were treated with ^{125}I-monotherapy, 33% with ^{103}Pd-monotherapy and 7% with a combined partial ^{103}Pd-implant and supplemental EBRT. Patients treated with a full ^{125}I-implant had a 71% (56/79) potency rate, while those treated with a full ^{103}Pd-implant had a 63% (27/43) potency rate, as evaluated by the MSEF score ($P = 0.36$) (Table 3). The treatment strategy, which incorporated EBRT and the partial ^{103}Pd-implant with 9 months of hormone therapy, maintained EF in three of the nine men. Of interest, among patients treated with ^{125}I- or ^{103}Pd-implants alone, no D90-related dose relationship was associated with the onset of ED. As expected, PSA failure was a strong predictor of ED among the present patients because of the use of either intermittent or continuous hormone therapy. Of the 131 patients, 23 had PSA failure and 15 (65%) of these developed ED; by contrast, 27 of 108 (25%) who had no PSA failure developed ED ($P < 0.001$).

Of patients who reported maintained EF after ≥7 years of follow-up 45/89 (51%) were currently using aids for EF, while six of 42 (14%) of those with erections insufficient for intercourse were using and aid for EF ($P < 0.001$; Table 4. Of these 51 patients, 44 (86%) were using either a phosphodiesterase type 5 (PDE-5) inhibitor, yohimbine (two, 4%) or alprostadil (five, 10%) at the final follow-up. The mean age at implantation of those using the aid was 63 years, vs 66 years in those not doing so ($P = 0.06$). In addition, there was a trend to significance between the association of adjuvant hormone use, at 29 (57%) vs 33 (41%), and the use of an erectile aid at the final follow-up ($P = 0.08$). Also, most men using an aid (45/51, 88%) claimed to have a good response to their chosen therapy.

DISCUSSION

Brachytherapy and/or EBRT appear to maintain higher rates of EF than RP, even though patients treated with radiation are a mean of 6–8 years older [23,24]. The reports that describe promising rates of preservation of EF after surgery focus on the subgroup of men aged 50–59 years and with intact sexual function before bilateral nerve-sparing RP done by high-volume surgeons [25,26]. In the present study we showed that comparably young and potent men treated with brachytherapy have a 92% likelihood of maintained sexual function at ≥7 years after completing treatment, using a similar type of physician-assigned measure. Based on this finding it is reasonable to conclude, to an even greater extent, that the same physiological redundancy which allows for preservation of EF in the younger man after RP is also accessible to the young patient after radiotherapy.

FIG. 1. The percentage of patients with an MSEF score of 2 or 3 and IIEF-5 score of ≥16 after ≥7 years of follow-up, and who had normal EF before brachytherapy.



Among men with no prostate cancer and aged >70 years the incidence of moderate to complete ED is about half [11]. This, in addition to the dramatic influence of age in this series, strongly suggests that ED after treatment for prostate cancer is multifactorial, with a strong dependence on both age and sexual motivation. In addition, it appears that younger patients are adequately treated with the current aids available for ED as it develops over the years after brachytherapy. Therefore, it is reasonable to hypothesise that the practice of supplying all patients treated with brachytherapy for prostate cancer with prophylactic PDE-5 inhibitors is not necessary in younger men. In the formulation of future trials to test prophylactic PDE-5 inhibitors, efforts should be made to target the more elderly patients who do not appear to benefit to the same extent as the younger patients from on-demand PDE-5 inhibitors when ED develops later in the follow-up.

The EF data were analysed by using both the patient-reported IIEF-5 and the physician-reported MSEF, which is based on the scale used in the Massachusetts Men's Aging study. While patient reported data are preferable, the IIEF-5 was only validated in 1999 [20]. Therefore, long-term data before and after treatment using only the IIEF-5, with an extended follow-up, was not possible in the present patients. However, in the present study, a significant percentage of patients classified as potent using the MSEF scale were classified as having ED based on an IIEF-5 score of ≤16; the IIEF-5 was validated in patients without prostate cancer, who

TABLE 3 The characteristics of the 131 patients with optimal EF before implantation and who developed ED or not

Characteristic	ED	No ED	P
Mean (range) age, years	63.7 (50–78)	67 (57–78)	<0.001*
N (%)			
Hormone use	44/89 (49)	18/42 (43)	0.48†
Age at implant (MSEF score 2 or 3 at last follow-up), years			
50–59	23/25 (92)	0/25	<0.001‡
60–69	48/75 (64)	0/75	
70–79	18/31 (58)	0/31	
Age at implant (IIEF-5 ≥ 16 at last follow-up), years			
50–59	16/25 (64)	0/25	<0.001‡
60–69	20/75 (27)	4/75 (5)	
70–79	6/31 (19)	1/31 (3)	
Isotope			
^{125}I	56/79 (71)	23/79 (29)	0.66‡
^{103}Pd	27/43 (63)	16/43 (37)	
partial	6/9	3/9	
Mean (SD, range) D90 ^{125}I, Gy	164.3 (35.5, (35.5–220)	136.0 (42.0, 55.8–206.3)	0.007*
Adjuvant hormones	13/56	3/23	0.31†
PSA failure on hormones	5/56	9/23	0.001†
Mean (SD, range) D90, ^{103}Pd, Gy, full implant	109.9 (25.1, (42.6–153.9)	103.1 (21.5, 76.8–145.6)	0.37*
Adjuvant hormones	25/27	12/16	0.11†
PSA failure on hormones	2/27	5/16	0.04†
Mean (SD, range) D90, ^{103}Pd, Gy, partial implant	81.0 (19.8, 56.6–107.4)	88.7 (14.6, 78.9–105.4)	0.55*
Adjuvant hormones	6/6	3/3	¶
PSA failure on hormones	1/6	1/3	¶
Use of drugs for ED§	45/89	6/42	<0.001†

**Student's t-test; Pearson's chi-square test, †2 × 2 table, 1 d.f.; ‡2 × 3 table, 2 d.f.; ¶sample too small for statistical validity. §Sildenafil, tadalafil, vardenafil, yohimbine, alprostadil.*

TABLE 4 Distribution of clinical characteristics between 131 patients who developed ED and those actively using aids for EF, and for the 89 patients who developed ED and reported maintained potency without the use of an erectile aid (44) or with an erectile aid (45)

Characteristic	Aids	No aids	P	Aids	No aids	P
N	51	80		45	44	
Mean (range) age, years	63 (50–78)	66 (51–78)	0.06*	63 (50–78)	64 (51–75)	0.45*
N (%)						
Hormone use	29 (57)	33 (41)	0.08†	27 (60)	17 (38)	0.04†
PSA failure	8 (15)	15 (19)	0.65†	5 (11)	3 (7)	0.48†
MSEF score at last follow-up visit						
3, optimal	31	26	<0.001‡	31	26	0.34†
2, suboptimal	14	18		14	18	
1, insufficient	3	19				
0, no erection	3	17				
IIEF-5 at last follow-up visit						
≥16	20	22	0.16†	20	21	0.76†

**Student's t-test; Pearson's chi-square test, †2 × 2 table, 1 d.f.; ‡2 × 4 table, 3 d.f.*

presented only for consideration of erectogenic therapy; therefore a dramatic discordance between an IIEF-5 score and a physician-assigned score occurs in the less sexually motivated patient who is still physiologically able to have erections [20]. Also, there has been a 'stage migration' in EF in recent reports of sexual health after interventions for prostate cancer, due to the widespread adoption of oral PDE-5 inhibitors for patients with true or expected ED after therapy. Recent reports in urological oncology have begun to characterize patients using erectile aids as 'not having ED'. A recent example from investigators at the Cleveland Clinic showed that after a bilateral nerve sparing RP the preservation of EF was 76% with sildenafil [27]. This is in contrast to the historical experience, where only 10–30% of patients maintain EF after RP when assessed using patient-reported questionnaires

[8,10,23,24]. To compare series, this less purist approach might become the only practical way to compare the outcomes of treatment for ED among therapeutic methods as the understanding of erectile function advances. In addition, it is reasonable to anticipate that use of these heavily promoted medications will continue to be adopted by a growing segment of men with historically adequate EF. The implication is that maintaining the tenet that a definition of ED must be contingent upon erectogenic therapy will lead to a widening discordance in the future between any given patient's sexual performance and their physician's assessment of the effect of a treatment on his sexual function.

In conclusion, there is a very significant age effect mediating the development of ED in men after completing brachytherapy. The prevalence of the use of erectile aids is very high amongst younger men and its efficacy appears to be consistent even after ≥7 years of follow-up evaluation. In addition, young men (aged 50–59 years) fare particularly well in terms of maintained EF.

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CONFLICT OF INTEREST

Drs Cesaretti and Stock serve as consultants for C.R. Bard; Dr Stone has a financial interest in Prologics, Inc.

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Abbreviations: **RP**, radical prostatectomy; **EBRT**, external beam radiotherapy; **ED**, erectile dysfunction; **II(EF)**, International Index of (Erectile Function); **MSEF**, Mount Sinai EF (score).

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REPORT

*GENE*TIC *P*REDICTORS OF *A*DVERSE *R*ADIOTHERAPY *E*FFECTS: THE GENE-PARE PROJECT

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Purpose: The development of adverse effects resulting from the radiotherapy of cancer limits the use of this treatment modality. The validation of a test capable of predicting which patients would be most likely to develop adverse responses to radiation treatment, based on the possession of specific genetic variants, would therefore be of value. The purpose of the *Gene*tic *P*redictors of *A*dverse *R*adiotherapy *E*ffects (Gene-PARE) project is to help achieve this goal.

Methods and Materials: A continuously expanding biorepository has been created consisting of frozen lymphocytes and DNA isolated from patients treated with radiotherapy. In conjunction with this biorepository, a database is maintained with detailed clinical information pertaining to diagnosis, treatment, and outcome. The DNA samples are screened using denaturing high performance liquid chromatography (DHPLC) and the Surveyor nuclease assay for variants in *ATM, TGFB1, XRCC1, XRCC3, SOD2,* and *hHR21.* It is anticipated that additional genes that control the biologic response to radiation will be screened in future work.

Results: Evidence has been obtained that possession of variants in genes, the products of which play a role in radiation response, is predictive for the development of adverse effects after radiotherapy.

Conclusions: It is anticipated that the Gene-PARE project will yield information that will allow radiation oncologists to use genetic data to optimize treatment on an individual basis. © 2006 Elsevier Inc.

Genetic predictors, Adverse radiotherapy effects, Breast cancer, Prostate cancer.

INTRODUCTION

The term "adverse radiation effects" can generally be defined as undesirable clinical and physiologic responses secondary to radiation treatment. In an effort to balance the eradication of clonogenic tumor cells with minimization of damage to surrounding normal tissues, the mechanisms underlying adverse responses to radiation therapy have been studied by both basic scientists and clinicians (1–5). In this article, both the historical and current literature examining genetic factors in adverse radiation response will be reviewed. In addition, current efforts and techniques used in the *Gene*tic *P*redictors of *A*dverse *R*adiotherapy *E*ffects (Gene-PARE) project will be discussed as well as future directions for developing genetic predictors of radiation-induced morbidity.

GENETIC FACTORS AND RADIOSENSITIVITY

A variety of patient, tumor, treatment, cellular, and molecular factors contribute to the variability in severity of

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normal tissue reactions exhibited after radiotherapy. Patient characteristics including age, nutritional status, medications, body habitus, and coexisting morbidities such as diabetes or recent surgery all may contribute to radiation toxicity (6). Tumor-related factors such as size, histology, and tumor grade may also affect the reaction to radiotherapy. Variation in treatment-related parameters including treated volume, field size, anatomic prescription point, total dose, dose per fraction, and use of concomitant chemotherapy may also contribute to response heterogeneity. Because of the steep dose–response relationship for normal tissues, a small difference in dose could produce divergent outcomes (7, 8). In addition, it has been hypothesized that individual genetic variations may also influence the development of adverse radiation responses (9–14). Evidence in support of this theory was obtained through a study (15) that examined the incidence and time to development of radiation-induced telangiectasia in a cohort of breast cancer patients. A wide range of values was reported for this patient population despite uniform radiation treatment. Consistent with the results of previous analyses of radiotherapy patients (8, 16, 17) it was estimated that approximately 80% to 90% of the variability was attributed to deterministic effects, possibly arising from potential individual genetic differences, whereas only 10% to 20% of the variation resulted from stochastic events associated with the random nature of radiation-induced cell killing in addition to random variations in dosimetry and dose delivery.

EFFORTS TO DEVELOP PREDICTIVE ASSAYS FOR NORMAL TISSUE RADIOSENSITIVITY

The development of an *in vitro* radiosensitivity assay capable of predicting the extent of normal tissue damage in radiotherapy patients represents a long-sought goal (18). Despite limited success, the effort to achieve this objective continues because an assay capable of predicting susceptibility for the development of adverse radiation effects would allow customization of radiotherapy protocols on an individual basis. By doing so, it has been estimated that a significant improvement in the therapeutic index could be achieved (16, 19). This work is also reflective of the new era of "individualized" or "personalized" medicine (20–22). The goal is therefore to develop a robust, specific assay to enable individual dose adjustment based on the response of each patient to this test (16, 19, 23, 24).

Numerous assays have been proposed to provide the clinician with information that predicts the outcome after irradiation and thus guide treatment prescription, but none have become established in daily practice. Major difficulties limiting the success of these assays are lack of sensitivity and specificity, technical burden of the procedures, poor characterization of the assayed cells, and the complexity of normal tissue radiobiology (25).

Skin fibroblast SF_2 assays

Several studies have attempted to define the relationship between *in vitro* radiation response and clinically evident effects by correlating fibroblast radiosensitivity with the development of acute and late radiation damage. The underlying hypothesis of these studies is that genetic differences may account for much of the unanticipated severity of acute and chronic radiation reactions exhibited by some radiotherapy patients. Several studies have reported a correlation between dermal fibroblast radiosensitivity quantified by clonogenic survival assays, measuring the SF_2 (*i.e.*, the surviving fraction after exposure to 2 Gy of X-rays), and the severity of both early and late effects (26, 27). In addition, it has been reported that *in vitro* fibroblast proliferation postirradiation may be a useful predictor of wound-healing morbidity for patients with soft tissue sarcoma who received preoperative radiotherapy (28). However, in contrast to these positive results, several studies have reported a lack of correlation between dermal fibroblast SF_2 with either early or late skin reactions (29). Taken together, these studies indicate that skin fibroblast sensitivity correlates only weakly with assessment of radiation-induced skin injury.

Lymphocyte assays

For assays of normal tissue radiation response, blood is considered to be the tissue of choice because of the ease of collection in a standardized, patient-convenient manner. However, initial lymphocyte radiosensitivity studies (30–33) were disappointing with respect to experimental variation, which confuted the predictive power of this assay. Because the various lymphocyte cell types display different radiation responses, fluctuations in the relative frequency of lymphocyte types cause an apparent shift in radiosensitivity resulting in large experimental variation (30, 31). However, by taking into account cell-type specific radiosensitivities, it has been reported that CD4 and CD8 T-lymphocyte radiosensitivity can discriminate differences in radiation-induced cytotoxicity between individuals (32–36), although it is premature to use such an approach as a predictive assay.

Chromosomal aberrations and micronuclei

Additional attempts to find suitable assays include analysis of fibroblast chromosomal aberrations (37). However, this technique is time consuming and allows examination of only a limited number of cells. Thus, it is considered impractical for cell types that exhibit slow growth and low mitotic indices. Micronucleus induction analysis is another means of detecting chromosomal damage. Although this assay has a well-established role in genetic toxicology (38) as a means of biomonitoring human populations (39) and as a biologic indicator of radiation damage (40–42), efforts to predict radiosensitivity have been inconclusive (43, 44).

Molecular approaches

Despite multiple and various attempts to develop an assay capable of predicting which patients are susceptible to de-

veloping adverse radiotherapy effects, none of the assays examined to date has proved to be consistently sensitive and accurate for the prediction of side effects among patients receiving radiation (45). However, new technologies in molecular biology may promote novel strategies for developing a predictive assay with clinical applicability. The use of gene expression arrays that could predict the variation in normal tissue sensitivity to radiation among individuals based on the expression patterns of different genes is currently under investigation. Several studies have demonstrated the predictive power of pretreatment expression profiling for human tumors (46–51), but similar large-scale studies on normal tissues to assess the extent of radiation-induced toxicity have yet to be reported. In addition, a few studies have demonstrated meaningful correlations with morbidity by focusing primarily on cytokine responses (52). Another new molecular approach involves analysis of DNA end-binding complexes that form at DNA double strand breaks after irradiation. It has been reported that the levels of *ATM*-containing complexes correlated with cellular radiosensitivity as measured by the SF_2 (53). Although these new molecular approaches appear to be promising, it has not yet been determined whether any will have clinical applicability.

GOAL OF THE GENE-PARE PROJECT

To develop an alternative approach to establish an assay predictive of which patients are most likely to experience radiation-induced complications, a research program has been initiated to identify the genetic factors associated with clinical radiosensitivity. To achieve this goal, a broad international effort has been organized comprising investigators from radiation oncology departments in the United States, Israel, France, and Switzerland, to create the Gene-PARE project (Table 1). Through the studies currently active in Gene-PARE, more than 2000 radiotherapy patients will be screened for genetic variants. The primary objective of Gene-PARE is to establish the genetic alterations, the presence of which may confer increased susceptibility for developing an adverse response to radiotherapy. Although the subjects screened to date are primarily breast and prostate cancer patients, the Gene-PARE tissue biorepository is not exclusive to these two types of cancers as it is open to tissue samples from patients diagnosed with any form of cancer treated with radiation. For all patients accrued into Gene-PARE studies, a blood sample is obtained for lymphocyte isolation and DNA extraction. In addition, frozen lymphocytes from patients exhibiting clinical radiosensitivity or notable genetic characteristics have been used for EBV transformation to create permanent cell lines, which are being used in assays examining the functional significance of specific variants.

By identifying genetic factors associated with radiosensitivity, the goal of Gene-PARE is to develop a means to predict which patients are at increased risk for complications secondary to radiation treatment. In this sense, we are attempting to "pare away" those individuals from the general patient population who are most likely to experience pronounced radiation-induced normal tissue damage. Although these radiosensitive patients may be better suited to a surgical treatment approach, paradoxically these individuals could alternatively represent a subset of patients who are actually optimal candidates for radiotherapy, given that their cancers should harbor identical sequence alterations associated with radiosensitivity. This highlights the potential for radiotherapy dose modification, as radiosensitive tumors theoretically should require lower total treatment doses than their genetically nonvariant counterparts. Conversely, for the vast majority of patients who do not possess genetic variants associated with radiosensitivity, it may be possible to dose escalate and potentially achieve a larger number of cancer cures.

Inclusion of African-American patients

A unique feature of Gene-PARE that distinguishes it from its European counterpart, the Genetic Pathways for the Prediction of the Effects of Irradiation (GENEPI) project (10, 54), coordinated through the European Society for Therapeutic Radiology and Oncology (ESTRO), as well as the developing Japanese RadGenomics (55) and the British Radiogenomics: Assessment of Polymorphisms for Predicting the Effects of Radiotherapy (RAPPER) and Radiation Complications and Epidemiology (RACE) studies (54), is the inclusion of a substantial number of patients of African-American ethnicity. Based upon currently funded Gene-PARE studies, it is anticipated that at a minimum, approximately 500 African-American subjects will be screened for genetic variants associated with clinical radiosensitivity. Screening of these samples may allow identification of important genetic predictors specific for this population, as genetic alterations that contribute to enhanced radiosensitivity could differ among ethnicities. Initial results of Gene-PARE studies suggest that substantial differences exist between the genetic factors associated with the development of adverse radiotherapy effects for African Americans compared with variants correlated with radiosensitivity in the general population (56). This preliminary finding is consistent with accumulating pharmacogenomic evidence indicating that African Americans have a significantly different spectrum of polymorphisms in genes associated with drug metabolism compared with those in the general population (57).

DISTINCTION BETWEEN MUTATIONS, SNPs, AND RARE VARIANTS

Several semantic issues deserve mention. Throughout this review, the word "mutation" is generally avoided, as this term is often used to signify a particular DNA sequence variation that exerts a functional impact on the protein encoded by the gene. Instead, the term "single nucleotide polymorphism" (SNP) is used to indicate a sequence variation in which the less common or minor allele occurs at a population frequency $>1\%$ (58). The expression "rare vari-

ant" is used to mean a sequence variation for which the minor allele occurs with a frequency <1%. Hence, these terms refer only to the prevalence of a minor allele and do not imply whether a particular genetic variant possesses functional or pathologic significance. The terms "DNA sequence variation" or "genetic variant/alteration" are used to signify SNPs and rare variants. The use of "mutation" is limited to avoid any suggestion as to the functional impact on the protein encoded by a gene possessing a particular variant allele.

ROLE OF *ATM* IN CLINICAL RADIOSENSITIVITY

During the initial years of the Gene-PARE project, substantial attention was devoted to study of the *ATM* gene and its relationship to radiosensitivity, which has pioneered the way for examination of other genetic variations as predictors of adverse radiation responses. The ATM protein functions as a protein kinase involved in cellular stress responses, cell-cycle checkpoint control and DNA repair (59–62). Loss of these functions may subsequently lead to a diminished DNA repair ability and defective cell-cycle checkpoint control. The clinical association between patients producing nonfunctional ATM protein and the subsequent devastating responses to ionizing radiotherapy have been described (63, 64). In addition, cells derived from individuals who were heterozygous for a mutation in *ATM* exhibited a radiosensitivity intermediate between persons diagnosed with AT and those who were not *ATM* carriers (65–70).

The initial studies examining the role of *ATM* variants in clinical radiosensitivity failed to find a positive correlation between *ATM* mutation status and the development of enhanced normal tissue damage in breast cancer patients (71–75). However, all of these studies used a protein truncation test, which only detects genetic alterations that cause protein truncations. Subsequent to these reports, evidence was obtained that missense mutations, which result in amino acid substitutions rather than protein truncation, are more prevalent in cancer patients and therefore serve as a more appropriate type of DNA alteration to measure for ascertainment of *ATM* mutational status (76–78).

In the first Gene-PARE study examining the role of *ATM* mutations in susceptibility to radiotherapy-induced morbidity, 46 breast cancer patients were screened for *ATM* sequence variations (79). It was reported that 3 of 3 (100%) of the patients who developed a Grade 3/4 subcutaneous reaction, manifested as either fibrosis or soft-tissue necrosis, had *ATM* missense variants. In contrast, only 3 of the 43 patients (7%) who did not develop this form of severe toxicity harbored this type of *ATM* alteration. In a separate study, DNA samples isolated from 41 postmastectomy patients who were treated with either a hypofractionated or standard radiotherapy fractionation protocol were screened (80). Because many of these patients received a hypofractionated treatment, radiation-induced skin fibrosis was relatively common in this cohort. Based on a logistic regression model, a dose–response using the ED_{50} (*i.e.*, the dose that resulted in a 50% incidence of Grade 3 radiation-induced fibrosis) was generated for these patients. The findings of this study suggest a correlation between possession of the 5557 G→A variant in *ATM* and radiosensitivity as the ED_{50} for women who were carriers of this SNP was 52 Gy, compared with an ED_{50} of 61 Gy for patients who did not possess this genetic alteration. These results are consistent with those of Angele *et al.* (81), who found a significant association between homozygote carriers of the G→A transition at *ATM* nucleotide 5557 and adverse radiotherapy responses, as well as a separate study that reported a nonsignificant overrepresentation of the *ATM* 5557 A allele among breast cancer patients with marked alterations in breast appearance after postlumpectomy radiotherapy (82). In addition, an association was reported between this SNP and late morbidity in prostate cancer patients, although it did not achieve statistical significance because of the small sample size (83).

Further evidence supporting the relationship between *ATM* sequence variations and radiosensitivity has been obtained for prostate cancer patients treated with iodine-125 (^{125}I) brachytherapy (84). The samples for these patients were obtained from the Mount Sinai Prostate Cancer Patient Tissue Biorepository, which represents a critical resource for Gene-PARE. This biorepository maintains DNA and frozen blood lymphocytes derived from the approximately 2400 prostate cancer patients treated with radiotherapy and followed at this medical center over the past 15 years. A pilot study involving *ATM* screening reported that 10 of the 16 subjects (63%) shown to possess sequence variants exhibited at least one form of adverse response (defined as erectile dysfunction, late rectal bleeding, or severe urinary disturbance). In contrast, of the 21 patients who did not harbor an *ATM* sequence variation, only 3 (14%) manifested radiation-induced adverse responses. Nine of the patients with sequence alterations specifically possessed missense mutations, which encode for amino acid substitutions, and are therefore more likely to possess functional importance. In this group, 7 of 9 (78%) exhibited at least one form of adverse response. In contrast, among the 28 patients who did not have a missense alteration, only 6 (21%) displayed any form of adverse response to the radiotherapy.

ADDITIONAL RADIOSENSITIVITY CANDIDATE GENES UNDER STUDY

Although there is now evidence supporting *ATM* as a gene associated with clinical radiosensitivity, it is nonetheless likely that this is not the only gene the alteration of which is responsible for adverse radiotherapy responses. Additional radiosensitivity candidate genes that have been linked to enhanced radiation responses include *TGFB1, XRCC1, XRCC3, SOD2,* and *hHR21*. TGFβ1, the protein encoded by *TGFB1*, is a key cytokine involved with the regulation of cell growth and immunosuppressive activities.

It is also associated with the deposition of extracellular matrix proteins and plays a central role in radiation-induced fibrosis (85). The primary function of the XRCC1 protein is to coordinate the activities of the enzymes that perform base excision repair of radiation-induced damage. Cells lacking a functional XRCC1 protein have demonstrated a hypersensitivity to radiation (86, 87). XRCC3 is involved in recombinational repair of radiation-induced DNA double strand breaks (88). *SOD2* encodes the manganese superoxide dismutatse that represents an important line of cellular antioxidant defense against the reactive oxygen species induced by irradiation (89). *hHR21* is the human homolog of the yeast *rad21* (90) the encoded protein of which is involved with repair of DNA double stand breaks (91), sister chromatid cohesion, and apoptosis (92).

To summarize this work, a correlation between radiosensitivity and the presence of a Pro/Pro at codon 10 and the T/T genotype in position −509 of *TGFB1* has been reported (82, 93). A relationship has also been demonstrated between the *SOD2* codon 16 Val/Ala, *XRCC3* codon 241 Thr/Thr and *XRCC1* codon 399 Arg/Arg genotypes and an increased risk of radiation-induced fibrosis (94). Another study screened three SNPs in *XRCC1* and detected an association with radiosensitivity for patients possessing either the codon 194 Arg/Trp alone or in combination with the codon 399 Arg/Gln genotype (95). Finally, a T→C transition at position 1440 of the open reading frame of *hHR21* has been found in 6 of 19 radiation-sensitive cancer patients (96).

In aggregate, these studies support the general hypothesis that genetic factors play a significant role as predictors of adverse radiotherapy responses. It is also important to note that the postmastectomy radiotherapy breast cancer patients who were screened through Gene-PARE for *ATM* variants have also been examined for SNPs in the additional genes cited above (94). From the results obtained, it appears that susceptibility to the development of radiation-induced fibrosis depends critically upon the total number of genetic variants possessed rather than on any single genetic alteration or gene affected (80). These findings suggest that clinical normal tissue radiosensitivity should be regarded as a complex genetic trait that is dependent on the effect of multiple DNA sequence variants.

Cellular radiosensitivity and possession of genetic variants

The Human Genome Project is a well-publicized example of the increasing effort to unravel the genetic variation underlying complex diseases and traits by illustrating the genetic differences existing between individuals (97). The role of SNPs and rare variants, which constitute approximately 90% of naturally occurring sequence variations, is of particular importance (98–100). SNPs and rare variants are known to potentially affect phenotype, although they have often been regarded as genetic changes without functional significance. However, these sequence alterations may in fact have an important biologic impact as genetic variants located within regulatory regions could affect gene expression, whereas amino acid substitutions resulting from variants present in exons may alter protein function. Even SNPs present within noncoding regions could be of significance through their affect upon RNA stability or splicing mechanisms (58).

The "allelic architecture" of complex traits has received significant attention (101–104). Susceptibility to adverse radiotherapy responses can be conceptualized through the two competing theories for the genetic basis of complex traits (105). The first theory, the so-called "common disease/ common variant hypothesis," suggests that the inherited basis of complex traits is most likely the result of genetic variants characterized by relatively high allelic frequencies (106). According to this theory, common SNPs in a limited number of genes are responsible for the inheritance of complex traits. However, this approach to identify genes associated with complex traits has achieved only modest success. Therefore, the alternative "rare variant" hypothesis has been proposed, which suggests that a large pool of alleles is accountable for the development of complex traits (107). The most realistic model for complex genetic traits likely incorporates aspects of both theories, with predisposing alleles of varying population frequencies present in the same and different genes. The Gene-PARE project will not be limited by either of these theories, as the approach being used in the studies that constitute this project routinely involves screening the entire coding portion of each candidate gene.

A question also arises as to the types of mutations that may be associated with clinical radiosensitivity. The studies reporting the results of *ATM* screening lend support to an association between minor sequence alterations, such as SNPs and rare variants, with susceptibility to adverse effects of radiotherapy (79–84). In contrast, evidence has been provided (72, 75, 108, 109) that patients who were carriers of pathogenic truncating mutations, which are typically the type of mutation found in individuals with AT (110), appear not to have been radiosensitive. It is possible that the presence of a null mutation in one copy of the *ATM* gene does not confer clinical radiosensitivity, whereas possession of a functional but altered ATM protein may result in an increased risk for the development of an adverse response to radiation treatment.

Radiosensitivity and tolerance dose

The question may also be raised as to whether a small difference in cellular survival associated with possession of genetic variants that confers a relatively small increase in cellular radiosensitivity could account for an increased severity in radiation response. In fact, the performance of a simple calculation demonstrates that this is a likely outcome. For example, an SF_2 for cells from an individual not possessing variants associated with radiosensitivity may be 0.5, whereas for a person possessing genetic variants causing mild radiosensitivity, the SF_2 could be 0.3. Considering a protocol involving the use of 25 2-Gy fractions, at the completion of treatment, cellular survival would be approx-

imately 3×10^{-8} for normal patients whereas it would be 8×10^{-14} for patients possessing radiosensitivity alleles. This effectively represents the biologic impact of an 88 Gy total treatment dose for radiosensitive patients compared with 50 Gy for the patients not harboring such genetic alterations. This large biologically effective dose could certainly account for adverse effects from the radiation treatment. In fact, when taking into account the relatively steep increase in the complication curves for normal tissue responses and the practice of treating to normal tissue tolerance, only a small increase in radiosensitivity could result in a large increase in the probability of normal tissue radiation-induced toxicity.

It is also important to note that this small increase in radiosensitivity may be difficult to detect through routine cellular radiosensitivity studies, considering the limitations in accuracy and precision of *in vitro* assays. Thus, when taking into account the steep slope of the normal tissue dose–complication curves, it is likely that a relatively modest, and possibly undetectable effect upon protein function, resulting in mild cellular radiosensitivity, could still substantially increase the probability for an adverse clinical response. Thus it may prove difficult or impossible to detect through functional assays the impact of a genetic variant that causes clinical radiosensitivity.

Denaturing high-performance liquid chromatography and the Surveyor nuclease assay

The principal screening techniques for identification of genetic variants in the Gene-PARE project are denaturing high-performance liquid chromatography (DHPLC) and the Surveyor nuclease assay. These are both robust techniques that can be used to screen any gene in a large population for single nucleotide substitutions as well as small deletions and insertions (111–113). The main advantage of DHPLC lies in its rapid and accurate identification of polymorphisms and rare genetic variants in an automated fashion with a high level of sensitivity and specificity (114–122). The samples obtained through Gene-PARE are also being screened using a complementary methodology that uses Surveyor nuclease (Trangenomic, Inc., Omaha, NE), which is a mismatch-specific DNA endonuclease. It is a member of the CEL nuclease family of plant DNA endonucleases. Surveyor nuclease cleaves with high specificity at the 3′ side of any mismatch site in both DNA strands, including all base substitutions and insertion/deletions up to at least 12 nucleotides. When mutant and wild-type alleles are mixed, heated, and then cooled to form heteroduplexes, Surveyor nuclease cleaves the heteroduplex fragments. The cleavage products are then analyzed using the same HPLC platform used for DHPLC but performed under nondenaturing conditions. This assay is performed under high sensitivity conditions in which the DNA is stained with a fluorescent probe and detected using a fluorescence detector. Hence the use of this approach permits the recognition of certain variants that are difficult to identify using DHPLC, which may require samples to be run at multiple denaturing temperatures to be

Table 1. Gene-PARE studies

Funding agency	Treated cancer site	Country where patients are accrued	Specific targeted ethnic group	Period of study	Screened genes	Number of subjects to be screened	Adverse effects
DOD-BCRP	Breast	U.S.	African-American	2002–2006	*ATM*	150	Telangiectasia, fibrosis
DOD-PCRP	Prostate	U.S.	None	2004–2009	*ATM*	200	ED, UTM, proctitis
NY State Dept. of Health	Breast and prostate	U.S.	None	2005–2007	*ATM, TGFB1 XRCC1 XRCC3, SOD2, hHR21*	100	Telangiectasia, fibrosis, ED, UTM, proctitis
ACS	Prostate	U.S.	African-American	2005–2009	*ATM, TGFB1 XRCC1 XRCC3, SOD2, hHR21*	225	ED, UTM, proctitis
VA	Prostate	U.S.	None	2005–2010	*ATM, TGFB1 XRCC1 XRCC3, SOD2, hHR21*	350	ED, UTM, proctitis
Danish Cancer Society	Breast, head, neck	Denmark	None	2004–unlimited	*ATM, TGFB1 XRCC1 XRCC3, SOD2, hHR21*	41	Fibrosis, telangiectasia
Swiss Cancer League	Breast	Israel	None	2005–2006	*ATM*	150	Telangiectasia, fibrosis
Cohort	Breast, head, neck	Switzerland	None	2005–2006	*ATM, TGFB1 XRCC1 XRCC3, SOD2, hHR21*	28	Telangiectasia, fibrosis
	Breast	France, Switzerland	None	2005–2007	*ATM*	1012	Telangiectasia, fibrosis (concomitant letrozole therapy

Abbreviations: ACS = American Cancer Society; ED = erectile dysfunction; DOD-BRCP = Department of Defense Breast Cancer Research Program; DOD-PCRP = Department of Defense Prostate Cancer Research Program; Gene-PARE = *Genetic Predictors of Adverse Radiotherapy Effects*; UTM = Urinary Tract Morbidity; VA = U.S. Veterans Affairs Administration.

detected. A further advantage in the use of the Surveyor nuclease assay is that it provides information not only as to the presence of a genetic alteration, but also its relative position in the DNA fragment being analyzed (123–126). Although genotyping assays designed to detect common SNPs may be less costly to perform, these assays are limited to detection of already known SNPs and are not designed to discover new sequence variants. Of greatest importance, DHPLC and the Surveyor assay are capable of detecting virtually all variants in a gene, rather than just specific SNPs.

CONCLUSION

The goal of the Gene-PARE project is to identify the genetic sequence variants that are predictive for the development of adverse effects resulting from radiotherapy. To accomplish this objective, a clinical database and biorepository of frozen lymphocytes derived from cancer patients treated with radiation have been established. DNA isolated from each tissue sample is being screened for variants in genes associated with radiation responses. It is expected that the results of Gene-PARE will enable the greater use of data generated as part of the Human Genome Project and the emerging field of radiogenomics. In addition, Gene-PARE will enable radiation oncologists to take greater advantage of the increasingly powerful and inexpensive methodologies to sequence DNA in anticipation of the day when patients diagnosed with cancer arrive at their initial radiation oncology consultation armed with their full genome sequenced (127, 128). By identifying genetic predictors of radiosensitivity, Gene-PARE may help cancer patients avoid serious complications that lead to severe morbidity, or even mortality, arising from organ damage secondary to radiotherapy. In addition, it could be discovered through this work that there exists a small radiosensitive portion of the population and that standard treatment doses are effectively being limited by their radiation tolerance. If these individuals can be identified through genetic screening, it may then be revealed that the vast majority of people are more resistant to radiation than generally assumed. This finding might permit radiation oncologists to be more aggressive and to dose escalate, which could translate not only into improved clinical outcomes for radiotherapy patients but also to more frequently provide safe treatment of relatively radioresistant cancers. Thus, the results of the research conducted under Gene-PARE will help in the development of a predictive test that will provide individuals faced with a diagnosis of cancer, and to their doctors, critical information that is necessary to reach optimal treatment decisions.

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CLINICAL INVESTIGATION **Prostate**

LOW-DOSE RATE PROSTATE BRACHYTHERAPY IS WELL TOLERATED IN PATIENTS WITH A HISTORY OF INFLAMMATORY BOWEL DISEASE

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Purpose: We report on the follow-up of 24 patients with a prior history of inflammatory bowel disease (IBD) treated with brachytherapy for early-stage prostate cancer.

Methods and Materials: Twenty-four patients with a history of inflammatory bowel disease (17 with ulcerative colitis (UC), 7 with Crohn's disease [CD]) underwent prostate brachytherapy between 1992 and 2004. Fifteen patients were treated with I-125 implantation and 6 patients were treated with Pd-103 alone or in combination with 45 Gy external beam radiation. Charts were reviewed for all patients, and all living patients were contacted by phone. National Cancer Institute common toxicity scores for proctitis were assigned to all patients. Actuarial risk of late toxicity was calculated by the Kaplan-Meier method. Statistical analysis was performed using SPSS software. Follow-up ranged from 3 to 126 months (median, 48.5 months; mean, 56.8 months).

Results: None of the patients experienced Grade 3 or 4 rectal toxicity. Four patients experienced Grade 2 late rectal toxicity. The 5-year actuarial freedom from developing late Grade 2 rectal toxicity was 81%. At a median follow-up of 48.5 months, 23 patients were alive and had no evidence of disease with a median prostate-specific antigen for the sample of 0.1 ng/mL (range, <0.05–0.88 ng/mL). One patient died of other causes unrelated to his prostate cancer.

Conclusions: Prostate brachytherapy is well tolerated in patients with a history of controlled IBD. Therefore, brachytherapy should be considered a viable therapeutic option in this patient population.

Prostate cancer, Brachytherapy, Inflammatory bowel disease, Radiotherapy.

INTRODUCTION

Inflammatory bowel disease (IBD) is a general term for two chronic inflammatory disorders of the gastrointestinal tract, ulcerative colitis (UC), and Crohn's disease (CD). UC is characterized by recurrent bouts of inflammation limited to the superficial mucosa of the colon. UC almost always involves the rectum and may extend continuously to involve more proximal portions of the large intestine. CD is characterized by transmural disease and can involve any portion of the gastrointestinal tract. Approximately 80% of CD patients have small bowel disease, most commonly in the distal ileum. Contrary to UC, roughly half of CD patients have no involvement of the rectum. Perianal involvement is common and the disease is characterized by skip lesions. Deep ulcerations, fistulas, and perforations often complicate CD. Both UC and CD are predisposing risk factors for the development of gastrointestinal malignancy (1).

Because both diseases manifest themselves as inflammatory reactions of the mucosa, it has been thought that the rectal inflammation caused by radiotherapy would exacerbate their baseline condition. Therefore, a history of IBD has been considered a relative contraindication for the administration of external beam radiotherapy (EBRT) to the pelvis (2). Because of this concern, patients with IBD and prostate cancer are often not considered to be candidates for radiotherapy management options. This situation is complicated by the fact that many IBD patients have undergone prior serious abdominal or pelvic surgery. At Mount Sinai, because of these concerns, patients with IBD have been offered brachytherapy for management of their prostate cancer. Brachytherapy delivers less radiation dose to the rectum and bowel than EBRT and should therefore cause fewer and less significant lower gastrointestinal symptoms in patients with IBD. There are few reports on the brachytherapy management of prostate cancer in patients with a history of IBD. The largest series reported on 6 IBD patients undergoing I-125 brachytherapy for localized clinical stage T1c-T2c adenocarcinoma of the prostate between 1991 and 1996. The median follow-up was 3.7 years. In this series,

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none of the 6 patients had significant gastrointestinal side effects after brachytherapy (3).

Because of the relative paucity of data regarding the safety of brachytherapy for prostate cancer patients with a history of IBD, we reviewed our database of prostate cancer patients with a history of IBD treated with brachytherapy at Mount Sinai Medical Center. In this report, we analyze the largest series of IBD patients with prostate cancer treated with brachytherapy to determine the treatment efficacy and treatment-related gastrointestinal toxicity seen in this special patient population.

METHODS AND MATERIALS

From a database of 2,500 patients treated with brachytherapy between 1990 and 2005, 25 patients were identified with a history of IBD. Detailed follow-up information was available for 24 of the 25 patients, with 1 patient lost to follow-up after implantation. As such, 24 patients were included in this analysis. The hospital charts, pathology reports, radiation therapy charts, and outpatient records were reviewed. In general, patients were seen in clinical follow-up every 6 months for the first 5 years posttreatment, then annually. All living patients were contacted by phone to obtain additional follow-up information. The diagnosis of IBD was established in all patients by clinical features, radiologic studies, endoscopy, and histologic examination of tissue biopsies of resected specimens. The diagnosis of prostate cancer was histologically confirmed by one pathologist with expertise in prostate cancer. At the time of consultation, patients stated they were not experiencing a flare in their IBD symptoms. Specifically, patients denied tenesmus, rectal bleeding, and change in their baseline number of daily bowel movements. Eleven of the 24 patients were taking baseline IBD medications at consultation. Nine patients had prior surgery for their IBD.

Of the 24 IBD cases identified, 17 had a history of UC and 7 had a history of CD. All patients had clinical stage T1c-T2b adenocarcinoma of the prostate using the 1992 American Joint Committee on Cancer staging. A breakdown of patients' prostate cancer risk stratification can be found in Table 1. Low risk was defined as follows: prostate-specific antigen (PSA) ≤10 ng/mL, Gleason score ≤6, and stage ≤T2a. Intermediate risk was defined as possessing only one of the following features: PSA >10–20 ng/mL, Gleason score = 7, or stage = T2b. High risk included those with two or more intermediate risk factors or one or more of the following features: PSA >20 ng/mL, Gleason score ≥8, stage T2c-T3. Detailed information on the location and extent of the IBD was available in 20 of the 24 patients and is listed in Table 2.

All patients were treated with brachytherapy using a real time ultrasound-guided technique (4). Treatment regimens developed over time so there was overlap in different risk groups being treated by different treatment regimens. Details of the development of these treatment schemas have been previously described. The implant prescription dose was 160 Gy (TG 43) for I-125 implants, 124 Gy (NIST 99) for full Pd-103 implants, and 100 Gy (NIST 99) for partial Pd-103 implants. Generally patients at higher risk for extracapsular extension based on pretreatment risk factors underwent partial (67%) dose implantation followed by EBRT. Three patients were treated with partial (67%) Pd-103 implants followed by EBRT to the prostate and seminal vesicle to a dose of 45 Gy using high-energy, 16-MV photons. All patients underwent computerized tomography–based postimplant dose evaluation at 1 month. In an effort to compare different treatment regimens (i.e., combined implant with EBRT) and different isotopes, we calculated biologic effective doses (BED) for the sample in a method previously described (5).

Table 1. Patient characteristics

	Ulcerative colitis 17 (71%)	Crohn's disease 7 (29%)	Total 24
Low risk	8	3	11
Intermediate risk	8	4	12
High risk	1	0	1

Table 2. Inflammatory bowel disease location

Ulcerative colitis	17	Crohn's disease	7
Rectum	8	Small bowel	3
Rectosigmoid	6	Ileocecal area	1
Entire colon	1	Rectovesicular fistula	1
No data	2	No data	2

Preimplant and postimplant rectal bleeding and change in bowel habits were evaluated for all patients. Radiation complications were evaluated at follow-up visits and scored using the National Cancer Institute (NCI) common toxicity criteria for proctitis to identify other symptoms such as tenesmus, increased stool frequency, or mucus discharge. The NCI common toxicity criteria are as follows: 0 = baseline; Grade 1 = increased stool frequency, occasional blood streaked stools, or rectal discomfort not requiring medication; Grade 2 = increased stool frequency, bleeding, mucus discharge, or rectal discomfort requiring medication, anal fissure; Grade 3 = increased stool frequency/diarrhea requiring parenteral support, rectal bleeding requiring transfusion, or persistent mucus discharge, necessitating pads; and Grade 4 = perforation, bleeding, or necrosis or other life threatening complication requiring surgical intervention. Side effects occurring within 6 months of treatment were characterized as acute effects and those occurring after 6 months as late effects. Follow-up was measured from the date of last irradiation (implant or EBRT) to the date of last contact. Follow-up ranged from 3 to 126 months (median, 48.5 months). Actuarial risk of late toxicity was calculated by the Kaplan-Meier method. Statistical analysis was performed using SPSS software.

RESULTS

At a median follow-up time of 48.5 months, 23 of the 24 patients were alive. One patient died of causes other than prostate cancer with his last PSA <0.05 ng/mL. All living patients had no evidence of disease (NED) with the median PSA for the population was 0.1 ng/mL (range, <0.05–0.88 ng/mL). Twenty-two of 24 patients did not experience any rectal bleeding or change in rectal bleeding from their preimplant baseline. In an effort to quantify morbidity associated with brachytherapy, we retrospectively scored patients' complaints at follow-up using the NCI common toxicity criteria for proctitis based on detailed descriptions obtained during prospective follow-up evaluations. These

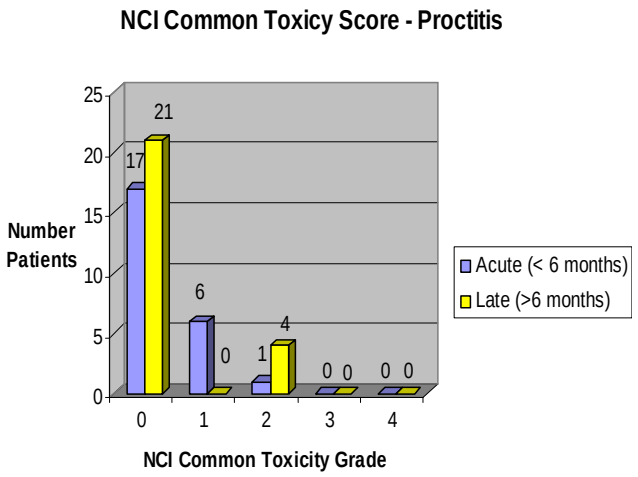

Fig. 1. National Cancer Institute common toxicity scores for proctitis. The vast majority of patients did not experience any rectal toxicity. There were no observed cases of late Grade 3 or 4 rectal toxicity. Four patients developed late Grade 2 rectal toxicity as described in the text.

data are reported in Fig. 1. None of the patients experienced Grade 3 or 4 early or late toxicity. Out of the 24 patients, 6 experienced Grade 1 early toxicity and 1 patient experienced Grade 2 early toxicity. The patient with the acute Grade 2 toxicity developed tenesmus after implant that lasted 6 months postimplant and was managed with mesalamine rectal suppositories.

Four patients developed Grade 2 late rectal toxicity over the course of their follow-up. Patient 1 (CD ileocecal area) developed an anal fissure 22 months after I-125 implant for which he was given Botox injections. At last follow-up 25 months postimplant, he had no further episodes of rectal bleeding and was on no medications for his CD. The D90 to the prostate (the dose that 90% of the prostate receives, reported in Gy) for this patient was 192.35 Gy and the rectal V100 (the volume of the rectum that receives the prescription (100%) dose, reported in cubic centimeters) was 2.01 mL, which would be considered a high but acceptable rectal dose based on the work of Snyder *et al.* from our group (6). Patient 2 (UC diffuse) had an increase in stool frequency consisting of three or four stools per day starting approximately 1 month after implant and lasting longer than 1 year postimplant. He was managed conservatively with mesalamine and has no episodes of rectal bleeding. The D90 to the prostate was 190.80 Gy and the rectal V100 was 1.15 mL. Patient 3 (UC rectosigmoid) experienced an acute severe exacerbation of his IBD symptoms within 2 weeks of implant consisting of bloody diarrhea daily for 6 months, progressively decreasing to baseline at 1-year postimplant. He received an I-125 implant where the prostate D90 was 187.10 Gy and the rectal V100 was 0.54 mL. He had colonoscopy documented radiation proctitis at 20 months postimplant. He was followed closely over the next 52 months and currently has no rectal bleeding. His IBD is stable and he is maintained on mesalamine suppositories and balsalazide. His prostate cancer is clinically and biochemically NED. Patient 4 (UC rectum and colon) noted occasional rectal bleeding, approximately three times per month at 30 months post–I-125 implant. His mild symptoms were managed with mesalamine. The D90 to the prostate was 202.90 Gy and the rectal V100 was 1.44 mL. Currently, the patient is clinically and biochemically NED.

Because there was no observed late Grade 3 or 4 rectal toxicity, an actuarial analysis was performed on the sample to determine the time to development of late Grade 2 rectal toxicity. As seen in Fig. 2, the actuarial freedom from developing Grade 2 late rectal toxicity was 81% at 60 months. The mean V100 to the rectum was 0.996 mL. The median prostate D90 was 171.77 Gy for I-125 implants. The median prostate D90 for full Pd-103 implants was 129.80 Gy and for partial Pd-103 implants was 100.30 Gy. The

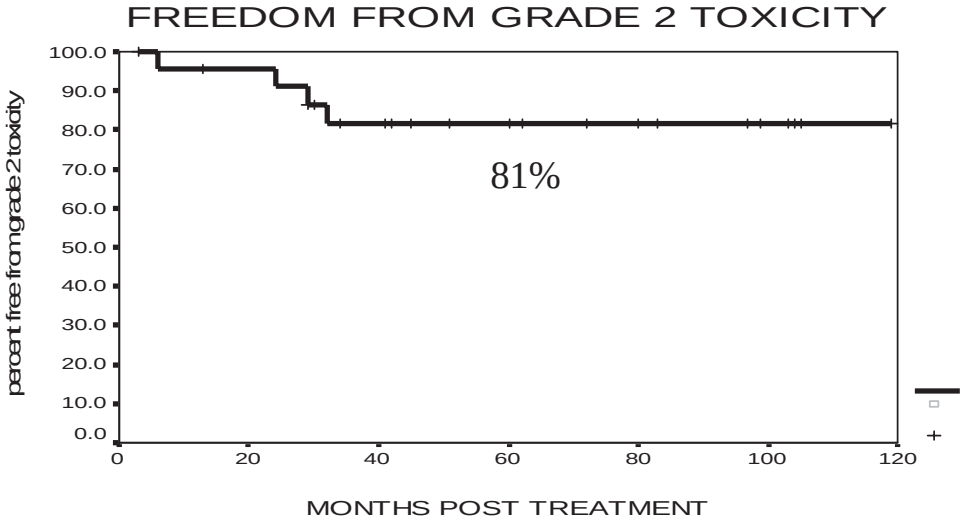

Fig. 2. Actuarial freedom from late Grade 2 rectal toxicity. The 5-year Actuarial freedom from developing late Grade 2 rectal toxicity is 81%. The median follow-up was 48.5 months (range, 3–119 months).

median BED for the population was 199 (range, 130–230). On univariate analysis, there was no significant association of BED and the development of late Grade 2 rectal toxicity ($p = 0.4$). Four patients in the sample had diabetes mellitus and 8 patients had a history of smoking. There was no significant association between having diabetes and smoking with the development of late Grade 2 rectal toxicity ($p = 0.8$ and 0.37, respectively).

DISCUSSION

A potential side effect of both external beam pelvic irradiation and prostate brachytherapy is the development of acute or late rectal toxicity. Histologically, acute radiation injury to the large intestine and rectum is demonstrated primarily in the mucosa. Eosinophilic crypt abscesses are a feature of the acute radiation reaction and an eosinophilic infiltrate can be seen in the lamina propria. Downstream effects from the acute inflammation also occur, including elevation of proinflammatory eicosanoids including leukotriene B2, B4, and prostaglandin E2. Changes in cytokines transforming growth factor–β1, bFGF, interleukin-1β, and tumor necrosis factor-α have also been observed after radiation exposure (7).

In contrast, late radiation injury to the bowel is associated with effect throughout the bowel wall and most prominent in the submucosa. Chronic radiation injury is attributable primarily to fibrosis and vascular insufficiency via chronic ischemia. On microscopy, the submucosa is characterized by atypical fibroblasts and collagen proliferation. Atypical vascular changes may be present and small arteries may show hyalinized wall thickening with intimal foam cell proliferation. Clinically, telangiectatic vessels may be present. There may be focal areas of stenosis or ulceration, the mucosa can appear congested and pale. Telangiectasias and ulceration or fistula formation may occur.

There is ample evidence that microvascular damage plays an important role in radiation sequela. Changes in endothelial cell function and the establishment of local procoagulant factors appear to play an important role in the acute radiation response (8). Additional support for the role of vascular dysfunction is observed in that diabetics are at increased risk for radiation-induced rectal injury (9).

There are substantial data in the external beam radiation literature relating the risk of radiation-induced proctitis to both the dose used for prostate therapy and the volume of rectum incidentally treated (10–12). From these series, one can conclude a 10–26% rectal bleeding rate can be expected for patients treated with EBRT who do not have a history of inflammatory bowel disease. The Radiation Therapy Oncology Group has conducted a Phase I-II dose-escalation trial using three-dimensional conformal radiotherapy for localized prostate cancer using a variety of different dose levels and fractionation with the primary endpoint being the development of Grade $\geq$3 late toxicity (13–17). The Grade $\geq$2 toxicity observed in those receiving 74 Gy in 2 Gy fractions was 19–23% at 2 years compared with a 9–13% incidence in those receiving 79.2 Gy in 1.8 Gy fractions, suggesting that a higher dose per fraction may be associated with a greater increase in late rectal toxicity (14).

The incidence of rectal toxicity in patients receiving brachytherapy for prostate cancer is also well described. Wallner described increased risk of proctitis correlating to area of rectal wall irradiated in patients receiving >100 Gy from I-125 brachytherapy (18). Our group has previously defined the risk of developing Grade 2 proctitis after I-125 brachytherapy using a rectal dose–volume histogram analysis. The study included 212 patients with T1-T2 prostate adenocarcinoma undergoing brachytherapy alone for definitive therapy with the prescription dose of 160 Gy. A dose–response analysis was performed for volumes of rectal tissue receiving a given dose. Proctitis was found to be significantly volume dependent for a given dose using iodine-125 implants. The prescription dose delivered to <1.3 mL of rectal tissue resulted in a 5% risk of proctitis at 5 years vs. 18% for volumes >1.3 mL ($p = 0.001$). In the dose–volume histogram analysis, the proctitis rate increased as the rectal volume receiving the prescription dose increased. The proctitis rates were: 0% for $\leq$0.8 mL, 7% for >0.8–1.3 mL, 8% for >1.3–1.8%, 24% for >1.8–2.3 mL, and 25.5% for >2.3 mL ($p = 0.002$) (6).

There is considerable hesitation to use pelvic irradiation in patients with inflammatory bowel disease for fear of increased bowel toxicity. It is possible that a chronically inflamed bowel would suffer increased risk of radiation-induced damage. Willett reported a series of 28 patients with IBD treated with abdominal or pelvic external beam radiation between 1970 and 1999 at Massachusetts General Hospital. In this series, acute effects were defined as severe if the patient did not complete the planned course of therapy because of enteral toxicity. Late effects were scored as severe if the patient required hospitalization or laparotomy after EBRT because of small or large bowel complications. Severe toxicity was seen in 13/28 patients (46%) overall. Six of 28 patients developed severe acute toxicity and 8 of 28 patients developed severe late toxicity. Specialized techniques to minimize bowel dose included specific radiation techniques such as small fields, decubitus position, proton beam, scheduled rest periods and surgical procedures including surgical clips, omentoplasty, or Dexon mesh. The 5-year actuarial rate of severe late toxicity was 73% in the conventional approaches group and 23% in the specialized techniques group ($p = 0.02$) (2). The authors concluded that the use of EBRT in patients with IBD must be judicious because of the potential risk of severe toxicity and that careful irradiation technique may allow treatment of these patients with acceptable morbidity.

In the largest series discussing patients with IBD and rectal cancer, our group has previously examined 47 patients treated between 1960 and 1994 for all stages of rectal cancer. Three of the 15 (20%) patients who received irradiation experienced acute Grade 3-4 toxicity, consisting of 2 patients with Grade 3 skin toxicity and 1 patient (6%) with

Grade 4 gastrointestinal toxicity requiring a 4-week treatment break and a short-term hospitalization for dehydration. There were no long-term complications in the irradiated patients. The toxicity of treatment, as well as overall survival, disease-free survival, and pelvic control rates were similar to that of rectal cancer patients in the general population and in reported Phase III trials (19–22). Song has reported a series of 24 patients with IBD treated with radiotherapy for various malignancies and found a 21% rate of Grade $\geq$3 acute gastrointestinal toxicity. All these patients were receiving concurrent chemotherapy. Two of 24 patients developed small-bowel obstructions, and both these patients had a history of total colectomy (23). These studies suggest that the risk of gastrointestinal complications is generally more modest than is perceived and carefully planned radiotherapy can be used with acceptable morbidity and efficacy in this special population.

Brachytherapy is an attractive modality in patients with prostate cancer because of the potential physical sparing of dose to the rectum. This may even be more significant in prostate cancer patients with inflammatory bowel disease. In this series, we have provided detailed long-term toxicity and dosimetric data on this population. We have found that the side effects arising from brachytherapy are similar to patients without IBD. This is the largest report of IBD patients with prostate cancer undergoing prostate brachytherapy. Out of the 24 patients analyzed, only 2 had a change in rectal bleeding after brachytherapy. In addition, none of the patients developed Grade 3 or 4 early or late gastrointestinal toxicity. Only 1 (4%) patient experienced early Grade 2 toxicity and 4 patients (17%) developed Grade 2 late toxicity. The patient who developed significant early toxicity developed it at 2 weeks after the implant procedure with I-125, an isotope with a half-life of 60 days. The implication of the association in this patient is that a very small percentage of the total rectal dose was delivered in this short time and that it is unlikely in this patient that the IBD flare-up was related to the radiation procedure. The incidence of late prostate brachytherapy related rectal toxicity has been widely reported and generally estimated to be around 10% (24, 25). Our data of rectal toxicity in patients with IBD compare favorably with patients without IBD.

In summary, we feel that radiotherapy can be used effectively in patients with a history of IBD. Every effort should be made to minimize the dose to the adjacent normal tissue. In prostate cancer, this problem can be approached with the use of brachytherapy taking advantage of the rapid dose fall-off from each source. In this way, a high biologic dose can be delivered to the target and dose can be minimized to the adjacent rectum. It is our practice at Mount Sinai to recommend low-dose-rate brachytherapy as part of the treatment plan for all risk categories of prostate cancer. Our results have been published regarding biochemical NED rates and cause-specific survival that reaffirm our belief in aggressive treatment of this malignancy.

CONCLUSION

Prostate brachytherapy is generally well tolerated and appears to be safe in prostate cancer patients with a history of inflammatory bowel disease. The biochemical control and rectal toxicity are similar to patients without IBD. These patients will continue to require close collaboration between the radiation oncologist and gastroenterologist in the management of this unique problem. If the IBD is controlled, prostate brachytherapy is a viable therapeutic option and should be considered for this group of patients.

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CLINICAL INVESTIGATION **Breast**

POSSESSION OF *ATM* SEQUENCE VARIANTS AS PREDICTOR FOR LATE NORMAL TISSUE RESPONSES IN BREAST CANCER PATIENTS TREATED WITH RADIOTHERAPY

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Purpose: The *ATM* gene product is a central component of cell cycle regulation and genomic surveillance. We hypothesized that DNA sequence alterations in *ATM* predict for adverse effects after external beam radiotherapy for early breast cancer.

Methods and Materials: A total of 131 patients with a minimum of 2 years follow-up who had undergone breast-conserving surgery and adjuvant radiotherapy were screened for sequence alterations in *ATM* using DNA from blood lymphocytes. Genetic variants were identified using denaturing high performance liquid chromatography. The Radiation Therapy Oncology Group late morbidity scoring schemes for skin and subcutaneous tissues were applied to quantify the radiation-induced effects.

Results: Of the 131 patients, 51 possessed *ATM* sequence alterations located within exons or in short intron regions flanking each exon that encompass putative splice site regions. Of these 51 patients, 21 (41%) exhibited a minimum of a Grade 2 late radiation response. In contrast, of the 80 patients without an *ATM* sequence variation, only 18 (23%) had radiation-induced adverse responses, for an odds ratio of 2.4 (95% confidence interval, 1.1–5.2). Fifteen patients were heterozygous for the G→A polymorphism at nucleotide 5557, which causes substitution of asparagine for aspartic acid at position 1853 of the ATM protein. Of these 15 patients, 8 (53%) exhibited a Grade 2-4 late response compared with 31 (27%) of the 116 patients without this alteration, for an odds ratio of 3.1 (95% confidence interval, 1.1–9.4).

Conclusion: Sequence variants located in the *ATM* gene, in particular the 5557 G→A polymorphism, may predict for late adverse radiation responses in breast cancer patients. © 2007 Elsevier Inc.

***ATM* gene, Breast cancer, Radiotherapy, Late tissue response, Genetic variants.**

INTRODUCTION

Radiotherapy (RT) is a well-established component in the management of early-stage breast cancer (1). Although well tolerated by most patients, fibrosis and telangiectasia are potential side effects after RT completion (2–4). The development of these normal tissue reactions in breast cancer patients receiving adjuvant external beam RT demonstrates significant heterogeneity among individuals (5), which can be attributed to a variety of patient, tumor, cellular, and molecular factors. During the past several years, a burgeoning amount of evidence has suggested that individual genetic variations may also play a significant role in the development of adverse radiation responses (5–9). The involvement of genetic variants and radiosensitivity candidate genes in the development of adverse radiation responses is an active area of investigation (8).

It was first hypothesized >30 years ago that the product of the gene defective in the disease ataxia-telangiectasia (AT) plays an important role in the development of adverse radiation responses (10, 11). The protein encoded by the *ATM* gene, which is mutated in people with AT, serves as a protein kinase involved in cellular stress responses, cell cycle

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checkpoint control, and DNA repair (12–15). The loss of these functions can result in decreased DNA repair ability and defective cell cycle checkpoint control. Several clinical cases have been reported in which patients with AT demonstrated severe responses to ionizing RT (10, 11). Enhanced radiosensitivity in cells derived from people with AT, who possess two mutated copies of *ATM*, has also been demonstrated at the cellular level (16, 17). Cells derived from individuals heterozygous for an *ATM* mutation exhibited a radiosensitivity response intermediate between people diagnosed with AT and those who were not mutant *ATM* carriers (18–22).

Recent evidence that possession of *ATM* DNA sequence variants might be predictive of adverse RT responses has been reported (6, 9, 23, 24). In one study, DNA samples isolated from 41 postmastectomy patients who were treated with either hypofractionated or standard RT fractionation protocols were screened (9). Using a logistic regression model, a dose response using the median effective dose (ED_{50}) (*i.e.,* the dose that resulted in a 50% incidence of Grade 3 radiation-induced fibrosis) was generated for these patients. The findings suggested a correlation between possession of the 5557 G→A variant in *ATM* and enhanced radiosensitivity, because the ED_{50} for women who were carriers of this single nucleotide polymorphism (SNP) was lower (52 Gy) compared with the ED_{50} of patients who did not possess this genetic alteration (61 Gy). These results were consistent with those of Angele *et al*. (25), who found a significant association between homozygote carriers of the *ATM* 5557 G→A and adverse radiation responses, as well as with a separate study that reported a nonsignificant overrepresentation of this polymorphism among breast cancer patients with marked alterations in breast appearance after postlumpectomy RT (26). Similarly, an association between *ATM* sequence variants and adverse effects has been reported for prostate cancer patients treated with RT (24, 27). In contrast to these findings, one study (28) failed to establish a correlation between late subcutaneous toxicity after RT in breast cancer patients carrying *ATM* gene mutations. However, the clinical observations in that study were restricted to patients who primarily possessed truncation type mutations within AT. Hence, consideration was not given to the other types of *ATM* variants that could potentially affect the development of adverse radiation responses.

An important feature of our study was the inclusion of a substantial number of African-American patients. Efforts to identify genetic predictors of adverse radiation responses have previously focused primarily on white subjects. However, accumulating pharmacogenomic evidence has suggested that African-Americans have a significantly different spectrum of genetic variations in genes associated with drug metabolism compared with the general population (29).

METHODS AND MATERIALS

Patients

Peripheral venous blood samples were collected from 131 female patients with American Joint Committee on Cancer (30) Stage 0-II breast carcinoma who underwent breast conservation surgery and adjuvant RT between 1987 and 2004 at three tertiary referral centers (Mount Sinai Hospital, New York University Medical Center, and Yale-New Haven Medical Center). Of the total patient population, the results of 46 of the patients had been reported in our previous study (23). We updated the clinical information of these subjects. All patients underwent primary surgical resection by way of lumpectomy or quandrantectomy for histologically confirmed invasive or ductal carcinoma *in situ* of the breast. Negative surgical margins were obtained in all cases. The RT was delivered with 6-MV photons using either opposed tangential portals alone or three fields, including an additional supraclavicular portal if more than four axillary lymph nodes were involved. The whole breast doses ranged from 45 to 50.4 Gy. In addition, 82% of patients received an electron boost to the surgical bed designed to bring the dose to the tumor bed to ≥60 Gy. The electron energy used was typically 6–12 MeV and was prescribed to the 85% isodose line. Wedges were used for tissue compensation and to improve dose homogeneity. Before 2004, two-dimensional plans were constructed. Beginning in 2004, three-dimensional conformal plans were used. The dose inhomogeneity range was within <10% for most patients. During the follow-up examination, patients gave informed consent, and a blood sample was collected. The follow-up examinations were performed on a 6-month basis beginning 1 month after RT completion. The minimal period between RT completion and the first follow-up examination was 1–18 months. A retrospective chart review of all screened patients was performed, and morbidity data were collected from the follow-up notes by a radiation oncologist who was unaware of the genetic results. All patients had a minimal follow-up of 2 years (range, 2–16). Acute and late skin and subcutaneous tissue toxicity was graded according to the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer morbidity criteria (31). Late radiation reactions were defined as responses that were observed >3 months after the completion of RT. In contrast, early reactions represented responses that occurred either during the treatment course or within 3 months after RT completion. A summary of the patient characteristics is given in Table 1.

Denaturing high performance liquid chromatography analysis

DNA was isolated from peripheral blood lymphocytes and all 62 coding exons for the *ATM* gene. Additionally, the short flanking intron sequences were amplified for germ line mutations using polymerase chain reaction, as described previously (23). Genetic variants were identified using denaturing high performance liquid chromatography and compiled into a central database. Denaturing high performance liquid chromatography analysis was performed on a WAVE Nucleic Acid Fragment Analysis System (Transgenomic, Omaha, NE) using buffer gradient and temperature conditions calculated using WAVEmaker software, version 3.3 (Transgenomic) designed for this purpose. Exons with an aberrant denaturing high performance liquid chromatography chromatogram underwent DNA forward and reverse sequencing using an ABI PRISM 377 DNA Sequencer (Foster City, CA).

Statistical analysis

Group comparisons were performed through a calculation of the odds ratios (ORs) with the 95% confidence intervals (CIs) (32). For univariate analysis of variables that might predict for late effects, the differences in proportions were derived using Fisher's exact *t* test. A two-sided *p* value of ≤0.05 was considered statistically significant.

Table 1. Patient demographics and tumor properties

Characteristic	% (*n*)
Age (y)	
<40	5 (7)
40–59	47 (61)
60–80	48 (63)
Ethnicity	
White	38 (50)
Black	37 (48)
Hispanic	21 (28)
Asian	4 (5)
Menopausal status	
Pre	24 (31)
Post	73 (96)
Peri	3 (4)
AJCC stage	
0	17 (22)
I	54 (71)
II	29 (38)
Histologic features	
DCIS	18 (23)
Invasive ductal	62 (81)
Invasive lobular	15 (19)
Other	5 (8)
Hormone receptor status	
ER/PR (+)	76 (99)
ER/PR (−)	22 (29)
Unknown	2 (3)
Diabetes	
Yes	32 (42)
No	68 (89)
History of smoking	
Yes	37 (49)
No	63 (82)

Abbreviations: AJCC = American Joint Committee on Cancer; DCIS = ductal carcinoma *in situ*; ER = estrogen receptor; PR = progesterone receptor.

RESULTS

Of the 131 screened patients, 51 (39%) possessed *ATM* sequence alterations located within exons or in short intron regions flanking each exon that encompass putative splice sites. A total of 30 different *ATM* variants were identified. The specific genetic variants identified in these patients and their corresponding amino acid substitution and normal tissue responses are listed in Table 2.

Of the 131 patients in the study population, 39 (30%) had a Grade 2 or worse late adverse response to RT, considered a clinically significant adverse response. Of the 51 patients who possessed an *ATM* variant, 21 (41%) developed a late response compared with 18 (23%) of the 80 patients who did not have an *ATM* alteration. Thus, possession of a sequence alteration in the *ATM* gene was associated with an OR of 2.4 (95% CI, 1.1–5.2) for the development of a Grade 2-4 late reaction to RT.

As reported in Table 3, of the 42 patients with a missense mutation (sequence variant that resulted in an amino acid substitution), 17 (40%) had a Grade 2-4 level late response compared with 22 (25%) of the 89 patients who did not possess a missense mutation (OR, 2.1; 95% CI, 0.9–4.5). When the analysis was performed for the 17 patients who possessed multiple variants, 10 (59%) demonstrated a Grade 2-4 late response compared with 29 (25%) of the 114 patients who did not possess multiple *ATM* variants (OR, 4.2; 95% CI, 1.5–12.0). Except for the 5557 G→A and 378 T→A polymorphisms, no other variants appeared in >5% of the subjects, making it impossible to examine whether a correlation existed between any other specific variant and radiation toxicity. Of the 15 patients with the 5557 G→A polymorphism, 8 (53%) developed Grade 2-4 late responses. In contrast, only 31 (27%) of the 116 who did not have this variant developed Grade 2-4 late toxicity (OR, 3.1; 95% CI, 1.1–9.4). A significant correlation was not detected between possession of the 378 T→A and late reactions.

The effects of total dose (>5,040 cGy), diabetes, a history of smoking, use of hormonal therapy, and Grade 2-4 acute effects were analyzed separately in relation to the development of Grade 1-4 late effects. Univariate analysis demonstrated that the presence of any *ATM* sequence variant and Grade 2-4 acute effects were predictive for development of a late adverse response. None of the other independent variables achieved statistical significance (Table 4).

Of the 131 patients in our study population, 42 (32%) were African American. A significantly greater proportion of African-American patients possessed an *ATM* sequence alteration (66%) compared with patients outside this group ($p < 0.001$, chi-square test). This primarily resulted from the presence of the common 378 T→A polymorphism found in the African-American population. For the African-American patients, 12 (43%) of the 28 who possessed an *ATM* variant developed a late Grade 2-4 response compared with 3 (21%) of the 14 who did not harbor a variant in this gene (OR, 2.8; 95% CI, 0.6–12.1).

To test our hypothesis that possession of *ATM* variants predicts for the development of late Grade 2-4 adverse radiation effects in an independent cohort of patients, an analysis of the patient population ($n = 85$) excluding the original 46 patients reported in a previous study (23), was performed. The OR associated with the development of Grade 2 fibrosis in patients who possessed any *ATM* variant increased in this second group of 85 patients (OR, 3.1; 95% CI, 1.1–8.6) compared with that of the first group of 46 patients (OR, 2.0; 95% CI, 0.6–6.9). Other comparisons of the ORs for both patient populations are provided in Table 5.

DISCUSSION

The results of this study support the hypothesis that possession of variants in the *ATM* gene, particularly the 5557 G→A polymorphism, is associated with the development of late subcutaneous radiation toxicity resulting from standard RT. We found a statistically significant OR of 2.4 (95% CI, 1.1–5.2) and 3.1 (95% CI, 1.1–9.4) for the development of Grade 2-4 late responses for women with either any *ATM* variant or, specifically, the 5557 G→A polymorphism, respectively.

Table 2. *ATM* variants identified in 51 breast cancer patients and corresponding adverse radiation effects

Pt. no.	Nucleotide location	Codon	Mutation type	Amino acid change	Race	Acute reaction grade	Late reaction grade	Late reaction type
1	1176C→G	392	S	G→G	AA	2	0	NA
2	1810C→T	604	M	P→S	W	1	1	T
3	2119T→C	707	M	S→L	W	0	0	NA
4	2362A→C	788	M	S→R	H	1	1	F
	2362A→C	788	M	S→R				
5*	6088A→G	2030	M	I→V	H	1	3	F, T
6*	2442C→A	814	M	D→E	AA	1	2	F, T
	2572T→C	858	M	F→L				
7	2685A→G	895	S	L→L	AA	1	1	F
8	2572T→C	858	M	D→E	AA	1	0	NA
9	3161C→G	1054	S	P→P	AA	1	1	F
	378T→A	126	M	D→E				
10	1176C→G	392	S	G→G	AA	1	0	NA
	378T→A	126	M	D→E				
	1176C→G	392	S	G→G				
11*	4138C→T	1380	M	H→Y	AA	2	2	T
	378T→A	126	M	D→E				
12	4578C→T	1526	S	P→P	AA	1	1	F
	378T→A	126	M	D→E				
13	6176C→T	2059	M	T→I	AA	1	0	NA
14	5557G→A	1853	M	D→N	W	2	1	F
	378T→A	126	M	D→E				
	5557G→A	1853	M	D→N				
15*	IVS38-8T→C	NA	NA	NA	AA	2	2	F
16	4138C→T	1380	M	H→Y	AA	1	0	NA
	4138C→T	1380	M	H→Y				
17*	4400A→G	1467	M	D→G	AA	2	3	F, T
18	4258C→T	1420	M	L→F	H	0	0	NA
19	4578C→T	1526	S	P→P	W	1	1	F
20	5557G→A	1853	M	D→N	AA	1	1	F
21	5557G→A	1853	M	D→N	AA	1	1	F
22*	5557G→A	1853	M	D→N	W	1	2	F
23*	5557G→A	1853	M	D→N	W	1	2	F
24*	5557G→A	1853	M	D→N	W	0	2	F
25	5557G→A	1853	M	D→N	W	2	1	F
26	5557G→A	1853	M	D→N	H	1	0	NA
27*	5557G→A	1853	M	D→N	H	2	2	F
28*	5557G→A	1853	M	D→N	AA	1	2	F
29*	5793T→C	1931	S	A→A	AA	2	2	F
	735C→T	245	S	V→V				
	5557G→A	1853	M	D→N				
30*	7397C→T	4266	M	A→V	W	3	4	N
	IVS5-7C→T	NA	NA	NA				
31*	378T→A	126	M	D→E	AA	1	2	F
	IVS5-7C→T	NA		NA				
	378T→A	126	S	D→E				
32	4578C→T	1526	M	P→P	AA	1	1	F
33*	IVS62+8A→C	NA	NA	NA	H	1	2	F, T
34*	IVS62+8A→C	NA	NA	NA	H	1	2	F
35	1176C→G	392	S	G→G	H	3	1	F
36	5557G→A	1853	M	D→N	W	1	1	F
37	5558A→T	1853	M	D→V	W	1	0	NA
38	378T→A	126	M	D→E	AA	2	1	F
39	5557G→A	1853	M	D→N	H	3	1	F
	2614C→T	872	M	P→S				
40	2685A→G	895	S	L→L	AA	3	1	F
	378T→A	126	M	D→E				
41*	1176C→G	392	S	T→T	AA	3	2	F
	378T→A	126	M	D→E				
42	6176C→T	2059	M	T→T	AA	2	1	F
	5793T→C	1931	S	A→A				
43*	9200C→G	NA	NA	NA	AA	3	2	F
44	378T→A	126	M	D→E	AA	2	1	F

(*Continued*)

Table 2. *ATM* variants identified in 51 breast cancer patients and corresponding adverse radiation effects (*Continued*)

Pt. no.	Nucleotide location	Codon	Mutation type	Amino acid change	Race	Acute reaction grade	Late reaction grade	Late reaction type
45*	378T→A	126	M	D→E	AA	2	2	F
46	378T→A	126	M	D→E	H	2	0	F
47*	5557G→A	1853	M	D→N	H	2	2	F
	1636C→G	546	M	L→V				
48*	2614C→T	872	M	P→S	AA	3	3	F
49	IVS20+9	NA	NA	NA	AA	1	1	F
	378T→A	126	M	D→E				
	2614C→T	872	M	P→S				
50*	4578C→T	1526	S	P→P	AA	3	3	F
51	IVS62+8A→C	NA	NA	NA	W	2	0	NA

Abbreviations: Pt. no. = patient number; S = synonymous; AA = African American; NA = not applicable; M = missense; W = white; T = telangiectasia; H = Hispanic; F = fibrosis; N = necrosis; IVS = intron substitution.
* Patients with late Grade 2-4 response.

Approximately one-third of our study population were African-American women. We found a significantly greater incidence of *ATM* genetic variants in this population. Of the 30 different variants identified, only 4 were shared between the African-American patients and those outside this racial group. In particular, the T→A transversion polymorphism at nucleotide 378, which results in substitution of glutamate for aspartate at position 126 of the *ATM* protein, was found in 13 (31%) of the 42 African-American women. In contrast, only 1 (1%) of the 89 non–African-American women harbored this variant. The one woman for whom this polymorphism was detected who was not African-American had herself identified as Hispanic. This woman could also have reasonably been categorized as African-American. These results highlight the importance of screening for radiosensitivity genes among racial groups, because differential expression of genetic variants predictive for radiation-induced adverse effects might exist among different populations.

The OR for the development of late effects among the original group of 46 patients was 2.0 (95% CI, 0.6–6.9) and 2.6 (95% CI, 0.4–17.4) for the presence of either any *ATM* variant or the 5557 G→A variant, respectively. Thus, even though neither of these results was statistically significant because of the relatively small sample size for this population, the ORs that were obtained, particularly for the 5557 G→A variant, suggest a correlation between possession of *ATM* variants and the development of late effects. One point regarding this original population of 46 patients requires clarification. In the study published in 2002 (23), we reported only on ''novel *ATM* mutations'' detected in these patients, rather than on all genetic variants. During the early period of our work, a focus was placed on what appeared at the time to be rare genetic alterations. By analogy to patients with AT, it was assumed that the more common genetic alterations could not be associated with clinical radiosensitivity. However, with development of the HapMap project (33) and genome-wide SNP association studies (34–36), recognition is growing that common SNPs might represent important genetic variants that correlate with a particular phenotype, such as clinical radiosensitivity, as has been observed for other diseases/phenotypes (37–41). Moreover, some of the genetic variants we initially identified in breast cancer patients as ''novel'' have now been routinely detected and reported in subsequent studies screening various populations for *ATM* variants (42–48). Thus, several of the *ATM* variants that were characterized as novel and rare by Iannuzzi *et al.* (23) can no longer be labeled as such. Therefore, the total number and variety of *ATM* genetic variants identified in the first group of 46 patients was greater than the number of novel mutations reported in the original study and is

Table 3. Comparison of Grade 2-4 skin reactions among patient groups

Variant or class of variant	Acute skin reaction (%)	Odds ratio (95% CI)	Late skin reaction (%)	Odds ratio (95% CI)
Any *ATM* variant (+)	47 (24/51)	1.7 (0.9–3.6)	41 (21/51)	2.4 (1.1–5.2)
Any *ATM* variant (−)	34 (27/80)		23 (18/80)	
5557 G>A (+)	47 (7/15)	1.4 (0.5–4.2)	53 (8/15)	3.1 (1.1–9.4)
5557 G>A (−)	38 (44/116)		27 (31/116)	
378 T>A (+)	64 (9/14)	3.2 (1.0–10.2)	43 (6/14)	1.9 (0.6–5.9)
378 T>A (−)	36 (42/117)		28 (33/117)	
Missense variant (+)	47 (20/43)	1.6 (0.8–3.4)	40 (17/42)	2.1 (0.9–4.50)
Missense variant (−)	35 (31/88)		25 (22/89)	
Multiple variants (+)	56 (10/18)	1.7 (0.9–3.6)	59 (10/17)	4.2 (1.5–12.0)
Multiple variants (−)	36 (41/113)		25 (29/114)	

Abbreviation: CI = confidence interval.

Table 4. Univariate analysis of variables that might predict for RTOG/EORTC late effects

Variable	p*
Total dose >5,040 cGy	0.33
Race	0.56
Diabetes	0.41
Smoking	0.47
Acute effects (Grade 2-4)	0.02
Any *ATM* variant	0.05

Abbreviations: RTOG = Radiation Therapy Oncology Group; EORTC = European Organization for Research and Treatment of Cancer.

* Fisher's exact t test.

comparable to that found in the newer group of patients. It is more accurate to state that a total of 30 variants in 16 of the 46 subjects screened were identified in the first study, rather than just the 9 ''novel mutations'' originally reported in only 6 patients. For the new group of 85 subjects, the OR was 3.1 (95% CI, 1.1–8.6) and 3.7 (05% CI, 0.9–14.3) associated with the possession of either any *ATM* variant or the 5557 G→A variant. Therefore, the results for this second group of subjects, which essentially constitutes a validation study with a replication set of patients, have independently confirmed the conclusion of the first study, which was that possession of *ATM* variants correlates with the development of late effects in breast cancer patients.

A study was recently published by Andreassen *et al*. (49), in which they reported an inability to replicate in a cohort of 120 patients the results previously obtained for a group of 41 patients screened for genetic variants in *ATM* (9), as well as *TGFB1, SOD2, XRCC1*, and *XRCC3* (26, 50). Although they were unable to provide an explanation for the lack of reproducibility for their data, one important difference between their earlier work and the most recent study was that the DNA samples used for their initial research were isolated from cultured fibroblasts and the DNA samples used in the more recent work were derived from formalin-fixed paraffin-embedded tissue samples. It is possible that even a modest effect on SNP detection resulting from differences in DNA isolation methods could help explain their conflicting findings.

It is important to note certain additional aspects to our study. The decision to view Grade 2 fibrosis as a clinically relevant late adverse response to RT and to categorize those patients who developed this reaction as developing a late skin reaction were several fold. The first reason was that the number of patients who developed Grade 3 fibrosis was too small to yield meaningful results for the study if only these patients were labeled as having a late skin reaction. Second, we believe that Grade 2 fibrosis represents a clinically relevant morbidity that is clearly distinguishable from a lack of fibrosis. Thus, even though the endpoint used might have more limited clinical significance compared with higher grade adverse responses, it can still be valuable for the identification of variants associated with radiosensitivity. In addition, although the assignment of a grade to the radiation response was determined retrospectively from the follow-up notes, it is critical to note that the radiation oncologist making the grade assignments was unaware of the genetic results. Thus, no bias was present in the grade assignment related to the genetic status of the patients. Finally, the rate of Grade 2 late effects was high (30%) in our screened patient cohort, because an effort was made to specifically accrue patients

Table 5. Comparison of percentage of late grade 2-4 skin reactions in first vs. second study*

Patient population	Variant or class of variant	Late grade 2-4 skin reaction (%)	Odds ratio (95% CI)
Study 1	Any *ATM* variant (+)	50 (8/16)	2.0 (0.6–6.9)
	Any *ATM* variant (−)	33 (10/30)	
Study 2	Any *ATM* variant (+)	37 (13/35)	3.1 (1.1–8.6)
	Any *ATM* variant (−)	16 (8/50)	
Study 1	5557 G>A (+)	60 (3/5)	2.6 (0.4–17.4)
	5557 G>A (−)	37 (15/41)	
Study 2	5557 G>A (+)	50 (5/10)	3.7 (0.9–14.3)
	5557 G>A (−)	21 (16/75)	
Study 1	378 T>A (+)	50 (1/2)	1.6 (0.1–27.1)
	378 T>A (−)	39 (17/44)	
Study 2	378 T>A (+)	42 (5/12)	2.5 (0.7–9.1)
	378 T>A (−)	22 (16/73)	
Study 1	Missense variant (+)	43 (6/14)	1.3 (0.4–4.5)
	Missense variant (−)	38 (12/32)	
Study 2	Missense variant (+)	39 (11/28)	3.0 (1.1–8.4)
	Missense variant (−)	18 (10/57)	
Study 1	Multiple variants (+)	71 (5/7)	5.0 (0.8–29.4)
	Multiple variants (−)	33 (13/39)	
Study 2	Multiple variants (+)	50 (5/10)	3.7 (0.9–14.3)
	Multiple variants (−)	21 (16/75)	

Abbreviation: CI = confidence interval.

* Study 1 represents 46 patients reported on previously (23); Study 2 represents the additional 85 patients accrued after Study 1.

who had developed complications. However, this did not affect the results of this study, because patient accrual was clearly performed before the genetic analysis.

The topic of dose inhomogeneity, and its potentially confounding effect on the development of late adverse radiation effects in the screened population, deserves mention. We stated that the dose inhomogeneity range was <10% for most patients included in this study. However, it is possible that the variations in skin and subcutaneous late reactions seen in this cohort could be attributed, at least in part, to the dosimetric variations among patients. Therefore, the differences observed in the prevalence of late effects that correlated with the possession of particular sequence variants could also be a reflection of dose inhomogeneities, particularly considering the modest number of subjects possessing each type of variant. Thus, greater efforts will be made to obtain more detailed dosimetric information in future work.

The subtleties underlying the differential mechanism and function of the ATM protein in early and late tissues should also be addressed. Our data suggest that the appearance of Grade 2-4 acute reactions correlates with the development of late fibrosis, yet *ATM* SNPs predict for only late reactions. One would assume that if the possession of *ATM* variants increases the likelihood of developing late radiation reactions, an increase in severe early radiation responses might also be anticipated. However, the cellular targets and mechanisms involved in the etiology of early and late radiation-induced effects in the skin are quite different. In late-responding tissues, variants in the *ATM* gene could affect the function of the encoded protein in a particular fashion, and, to such an extent, that damage in subcutaneous tissue translates clinically into the development of adverse late reactions, such as fibrosis, which involves a cytokine cascade. In contrast, the role of the ATM protein is likely quite different in cells constituting the epidermis, in which early effects could be attributed primarily to cell killing, such that the small affect on ATM function caused by the presence of allelic variants might not be adequate to increase the probability for early radiation toxicity. It is, therefore, not altogether surprising to observe a differential impact of *ATM* variants in the development of early compared with late radiation effects. In addition, we emphasize that our hypothesis is not that variants in the *ATM* gene specifically lead to increased cellular radiosensitivity, but simply, that the presence of sequence variants located in *ATM* correlates with the development of late adverse radiation responses in breast cancer patients. The mechanism underlying the functional affect of these sequence variants and the development of late responses remains to be elucidated.

CONCLUSION

We report a correlation between the possession of variants in the *ATM* gene, particularly the 5557 G → A polymorphism, among breast cancer patients treated with RT and the development of adverse late subcutaneous normal tissue effects. However, it is important to recognize that our findings do not rule out the coexistence of alterations in additional genes that could also play a role in the development of adverse radiation responses in normal tissues. Variants in other genes, including *TGFB1, XRCC1, XRCC3, SOD2*, and *RAD21*, have also been recognized as potential predictors of adverse RT responses (50–53). An association has been reported between radiation-induced fibrosis and variants in the *ATM, TGFB1, SOD2, XRCC1*, and *XRCC3* genes for 41 patients treated with postmastectomy RT for breast cancer (9). That work demonstrated that the ED_{50} for Grade 3 fibrosis correlated with the total number of ''risk alleles'' harbored at six polymorphic sites in these genes. Taken together, we conclude that the combined influence of multiple genetic alterations could determine the clinical normal tissue radiosensitivity. Among the battery of genes tested in such an assay, the genetic variants in *ATM* will continue to play an important role as potential predictors for the development of adverse radiation reactions in breast cancer patients.

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CLINICAL INVESTIGATION

PROGNOSTIC SIGNIFICANCE OF 5-YEAR PSA VALUE FOR PREDICTING PROSTATE CANCER RECURRENCE AFTER BRACHYTHERAPY ALONE AND COMBINED WITH HORMONAL THERAPY AND/OR EXTERNAL BEAM RADIOTHERAPY

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Purpose: To analyze the prognosis and outcomes of patients who remain free of biochemical failure during the first 5 years after treatment.

Methods and Materials: Between 1991 and 2002, 742 patients with prostate cancer were treated with brachytherapy alone ($n = 306$), brachytherapy and hormonal therapy ($n = 212$), or combined implantation and external beam radiotherapy (with or without hormonal therapy; $n = 224$). These patients were free of biochemical failure (American Society for Therapeutic Radiology and Oncology [ASTRO] definition) during the first 5 post-treatment years and had a documented 5-year prostate-specific antigen (PSA) value. The median follow-up was 6.93 years.

Results: The actuarial 10-year freedom from PSA failure rate was 97% using the ASTRO definition and 95% using the Phoenix definition. The median 5-year PSA level was 0.03 ng/mL (range, 0–3.6). The 5-year PSA value was ≤0.01 in 47.7%, >0.01–0.10 in 31.1%, >0.10–0.2 in 10.2%, >0.2–0.5 in 7.82%, and >0.5 in 3.10%. The 5-year PSA value had prognostic significance, with a PSA value of ≤0.2 ng/mL ($n = 661$) corresponding to a 10-year freedom from PSA failure rate of 99% with the ASTRO definition and 98% with the Phoenix definition vs. 86% (ASTRO definition) and 81% (Phoenix definition) for a PSA value ≥0.2 ng/mL ($n = 81$; $p < .0001$). The treatment regimen had no effect on biochemical failure. None of the 742 patients in this study developed metastatic disease or died of prostate cancer.

Conclusion: The results of this study have shown that the prognosis for patients treated with brachytherapy and who remain biochemically free of disease for ≥5 years is excellent and none developed metastatic disease during the first 10 years after treatment. The 5-year PSA value is prognostic, and patients with a PSA value <0.2 ng/mL are unlikely to develop subsequent biochemical relapse.

Prostate cancer, Brachytherapy, 5-year PSA level, Biochemical failure, Outcomes.

INTRODUCTION

Newly diagnosed patients with prostate cancer are often presented with three National Cancer Care Network recommended primary therapeutic approaches: brachytherapy, external beam radiotherapy (EBRT), or surgery. The likelihood of success (biochemical control) as measured by prostate-specific antigen (PSA) follow-up for each treatment modality is considered to be equal within a few percentage points of uncertainty.

The PSA kinetics after prostate brachytherapy are often very difficult to interpret and can be a source of anxiety for both clinicians and patients. Unlike prostatectomy, in which a PSA level >0.2 ng/mL is considered failure, brachytherapy results in a gradual decline in the PSA level, with occasional increases in some individuals (1, 2).

It is, therefore, important to inform patients at what point they would be considered cured after brachytherapy. To address this issue, we analyzed the prognosis and outcomes for patients without a documented failure in the first 5 years after treatment. In particular, we were interested in the incidence of late biochemical failure and the prognostic significance of the 5-year PSA value for predicting future disease recurrence.

METHODS AND MATERIALS

Between July 1991 and February 2002, a total of 742 patients with Stage T1-T3 prostate cancer were treated with brachytherapy at Mount Sinai Hospital (New York, NY) and had a minimum of 5 years of follow-up with no evidence of PSA failure using the American Society for Therapeutic Radiology and Oncology

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(ASTRO) definition during their first 5 post-treatment years (3). Although a new definition for PSA failure has been recommended (the Phoenix definition), the ASTRO definition is still considered valid, provided sufficient follow-up is available (4). The ASTRO definition was chosen because all the patients in the study had sufficient follow-up (5-year minimum), and because it backdates the failure to the beginning of the increasing PSA profile. We believe this date signifies the start of the biochemical failure and is thus more reflective of the actual timing of the failure. Because the focus of this analysis was on the incidence of late failure, the use of the ASTRO definition would exclude patients who had an indication of an increasing PSA level during the first 5 years of follow-up. All patients underwent disease staging using the 1992 American Joint Committee on Cancer staging system, and no patient had radiographic or pathologic evidence of metastatic disease at presentation (5). The clinical presentation of all patients by Gleason score, PSA level, stage, and risk groups is given in Table 1.

The patients were divided into low-, intermediate-, and high-risk groups. Low risk was defined as a PSA level of ≤10 ng/mL, Gleason score of ≤6, and Stage T2a or less. Intermediate risk was defined as possessing only one of the following features: PSA level >10–20 ng/mL, Gleason score of 7, or Stage T2b. High risk included those with two or more intermediate-risk features or one or more of the following features: PSA >20 ng/mL, Gleason score of ≥8, Stage T2c-T3, or positive seminal vesicle biopsy findings. Seminal vesicle biopsy and laparoscopic pelvic lymph node dissection were done at the discretion of the urologist. A total of 337 patients underwent seminal vesicle biopsy, and 19 had positive findings; and 106 patients underwent laparoscopic pelvic lymph node dissection, and 2 had positive nodes.

Treatment

All patients were treated with brachytherapy using a real-time ultrasound-guided technique. The technique has been previously described (6). The treatment regimens were developed over time and were divided into three categories: brachytherapy alone (n = 306), brachytherapy and hormonal therapy (n = 212), and combined implantation and EBRT (with or without hormonal therapy; n = 224) (7).

Brachytherapy without EBRT (with or without hormonal therapy) was performed using both ^{125}I (prescription dose, 160 Gy, Task Group 43; 414 patients) and ^{103}Pd (prescription dose, 124 Gy, National Institute of Standards and Technology 1999 primary calibration standard; 328 patients). In general, ^{125}I was used for patients with a Gleason score of ≤6 and ^{103}Pd for those with a Gleason score of ≥7. Most patients treated with brachytherapy alone were low risk, although during the early years of the study period both intermediate- and high-risk patients received implantation alone.

Table 1. Disease characteristics

Factor	Patients (%)
PSA (ng/mL)	
≤10	528 (71)
>10–20	159 (21)
>20	55 (8)
Gleason score	
≤6	537 (72)
7	139 (19)
8–10	66 (9)
Clinical stage	
T2a or less	491 (66)
T2b	164 (22)
T2c or greater	85 (12)
Risk group	
Low	328 (44)
Intermediate	181 (24)
High	231 (32)

Abbreviation: PSA = prostate-specific antigen.

Hormonal therapy was used with brachytherapy for two main reasons. The first use of hormonal therapy was for downsizing in patients with large prostates (gland size, >50 cm^3). It was given for 3 months before implantation and usually for 2–3 months after implantation. The second use was as adjuvant therapy with brachytherapy for patients with intermediate- or high-risk features. In this case, hormonal therapy was given for 3 months before and 3 months after implantation (8).

Trimodality therapy usually involved 3 months of hormonal therapy followed by ^{103}Pd brachytherapy implantation (198 patients; prescription dose, 100 Gy, National Institute of Standards and Technology 1999 primary calibration standard) or ^{125}I (1 patient; prescription dose, 120 Gy) and 2 months later, EBRT to a dose of 45 Gy. The seminal vesicles were implanted in patients with biopsy-positive seminal vesicle disease. The total duration of hormonal therapy was 3–9 months (median, 9). In the earlier years of the study, lower implant doses were used with greater EBRT doses. The EBRT dose range was 39.6–61.2 Gy (median, 45). The details of this regimen have been previously described (9). The EBRT fields were conformal and treated the prostate and seminal vesicles using 1.5–2-cm margins. Overall, when hormonal therapy was used, it involved a luteinizing hormone-releasing hormone with or without an antiandrogen.

Dose equations

The dose delivered to the prostate was calculated with a 1-month postimplant computed tomography-based dosimetric analysis. All patients were asked to return 1 month after implantation for computed tomography scanning. Dosimetry was performed in 723 patients. The reasons for not performing dosimetry were poor visualization due to hip prostheses or patient noncompliance. The implant dose was defined as the dose delivered to 90% of the gland from the dose–volume histogram) (10). To compare the doses between the different isotopes and between implantation alone and combined implantation and EBRT, biologically effective dose (BED) equations were used. The BED values were obtained for both low-dose-rate permanent implants and the EBRT portions of the treatment. An α/β ratio of 2 was used in these equations. The details of these equations have been previously described (11). Patients treated with combined implantation and EBRT had their BED values for both methods combined to determine the total BED. The BED values for all treatments were 48–282 Gy2 (median, 195 Gy2).

Follow-up

All patients were asked to return every 6 months after treatment completion. Follow-up information was obtained from the clinical visits, telephone calls to referring physicians and patients, and mailed questionnaires. The follow-up blood tests included serum PSA and testosterone levels. Follow-up was calculated from treatment completion to the last available follow-up date or date of death. The follow-up period for the entire population was 5–14.4 years (median, 6.93). Survival curves were determined using the Kaplan-Meier methods. The freedom from biochemical failure rates were calculated using both the ASTRO and the Phoenix definitions. Distant metastasis was defined as radiographic or pathologically

determined evidence of disease outside the pelvis, including bone, visceral organ, or nodal disease. Differences in survival rates were calculated using the log–rank test. Differences in mean values were tested using the Student *t* test (12).

RESULTS

The actuarial freedom from PSA failure (FFPF) for the whole cohort at 10 years was 97% using the ASTRO definition and 95% using the Phoenix definition. In patients with treatment failure by either definition, the median PSA doubling time was 9 months (range, 1.7–54). The post-treatment follow-up testosterone levels for those initially treated with hormonal therapy and with no evidence biochemical failure by either definition were available for 265 patients. The testosterone level was 20–1,326 ng/dL (mean, 461); 88% had a testosterone level >200 ng/dL.

The FFPF rates stratified by presenting disease characteristics are listed in Table 2. No single presenting disease characteristic significantly affected the 10-year FFPF on univariate analysis. The 2 patients with positive nodes at lymph node dissection were treated with implantation and hormonal therapy and were free of biochemical failure at their last follow-up visit. In addition, the 19 patients with seminal vesicle biopsy-positive disease, who all met the initial criteria of no failure within the first 5 years, also were free of biochemical failure at their last follow-up visit. Table 3 lists the effects of the treatment regimen, isotope choice, hormonal therapy, and use of supplemental EBRT on biochemical failure. No significant effects were seen. Specifically, no difference was detected among the four different treatment groups. A pairwise comparison found no difference between the treatment groups of implantation alone vs. implantation and hormonal therapy (p = .35, ASTRO; p = .42 Phoenix), implantation alone vs. implantation and EBRT (p = .65, ASTRO; p = .5, Phoenix), implantation alone vs. implantation/EBRT/hormonal therapy (p = .2, ASTRO; p = .4, Phoenix), implantation and hormonal therapy vs. implantation and EBRT (p = .66, ASTRO; p = .64, Phoenix), implantation and hormonal therapy vs. implantation/EBRT/hormonal therapy (p = .62, ASTRO; p = .61, Phoenix), and implantation and EBRT vs. implantation/EBRT/hormonal therapy (p = .71, ASTRO; p = .76, Phoenix).

Table 2. Effect of prognostic factors on biochemical failure

	10-y FFPF (%)	
Factor	ASTRO	Phoenix
PSA (ng/mL)		
≤10	96.5	94
>10–20	99	98
>20	95	94
p	.4	.65
Gleason score		
≤6	97	96
7	97	91
8–10	100	100
p	.7	.8
Clinical stage		
T2a or less	97	94
T2b	97	97
T2c or greater	100	100
p	.3	.4
Risk group		
Low	96	92
Intermediate	98	99
High	99	97
p	.9	.7
Seminal vesicle biopsy status		
Not performed	97	94
Negative	98	96
Positive	100	100
p	.8	.6
Lymph node dissection status		
Not performed	98	97
Negative	95	92
Positive	100	100
p	.2	.5

Abbreviations: PSA = prostate-specific antigen; FFPF = freedom from PSA failure; ASTRO = American Society for Therapeutic Radiology and Oncology.

Five-year PSA groups

The 5-year PSA value was defined as the PSA value documented within a 1-year period around the 5-year follow-up date (6 months before the date to 6 months after). The PSA value closest to the 5-year follow-up date was selected. The median 5-year PSA level was 0.03 ng/mL (range, 0–3.6). For the purposes of this analysis, the patients were divided into 5-year PSA groups: PSA ≤0.01 ng/mL (n = 354), PSA >0.01–0.10 ng/mL (n = 231), PSA >0.10–0.20 ng/mL (n = 76), PSA >0.20–0.50 ng/mL (n = 58), and PSA >0.5 ng/mL (n = 23). The corresponding median follow-up for the 5 groups was 6.9, 6.4, 7.4, 6.9, and 7.5 years. These PSA groups significantly affected the FFPF rates (Figs. 1

Table 3. Effect of treatment on biochemical failure

		10-y FFPF (%)	
Factor	Patients (*n*)	ASTRO	Phoenix
Treatment regimen			
BT alone	306	95	93
BT + HT	212	99	97
BT + EBRT (no HT)	25	100	100
BT + EBRT (with HT)	199	99	98
p		.5	.7
Hormonal therapy			
No	331	95	94
Yes	411	99	97
p		.2	.45
EBRT			
No	518	97	95.5
Yes	224	99	98
p		.25	.8
Isotope			
^{125}I	415	96.5	95
^{103}Pd	327	98	97
p		.9	.6

Abbreviations: BT = brachytherapy; HT = hormonal therapy; EBRT = external beam radiotherapy; other abbreviations as in Table 2.

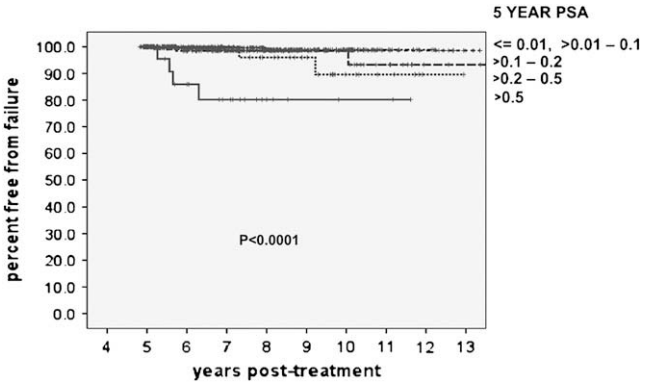

Fig. 1. Effect of 5-year prostate-specific antigen (PSA) level on freedom from PSA failure using American Society for Therapeutic Radiology and Oncology (ASTRO) definition.

and 2). The 10-year FFPF rates for the five groups using the ASTRO and Phoenix definitions were 99% and 97% for those with a 5-year PSA level of ≤0.01 ng/mL, 99% and 99% for PSA >0.01–0.1 ng/mL, 98.5% and 97% for PSA >0.1–0.2 ng/mL, 90% and 87.5% for PSA >0.2–0.5 ng/mL, and 80% and 65% for PSA >0.5 ng/mL, respectively ($p < .0001$). According to these survival curves for these groups, a separation was evident at a cutpoint of 0.2 ng/mL. Patients with a 5-year PSA level of ≤0.2 ng/mL ($n = 661$) had a FFPF rate at 10 years of 99% with the ASTRO definition and 98% with the Phoenix definition compared with a rate of 86% (ASTRO) and 81% (Phoenix) for patients with 5-year PSA level >0.2 ng/mL ($n = 81$; $p < .0001$; Figs. 3 and 4).

Improved BED and 5-year PSA values over time

The actuarial overall and disease-specific survival rate for the whole cohort at 10 years was 93% and 100%, respectively. The freedom from distant metastasis rate was also 100% for the entire cohort. These data suggest that biochemical failure ≥5 years after brachytherapy is likely due to the persistence of localized, indolent disease rather than aggressive or metastatic disease, and, thus, improvements in local

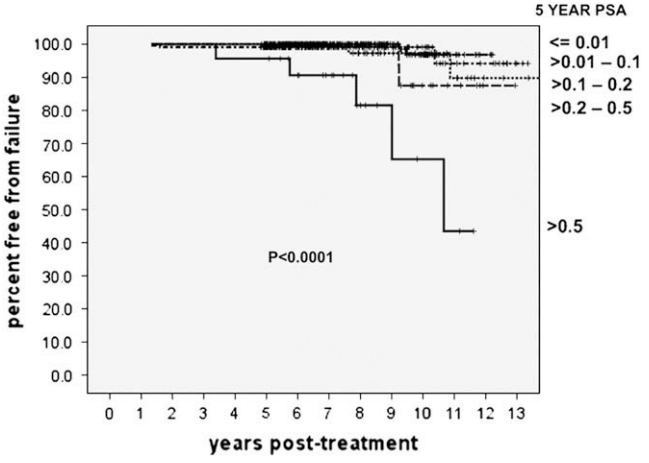

Fig. 2. Effect of 5-year prostate-specific antigen (PSA) level on freedom from PSA failure using Phoenix definition.

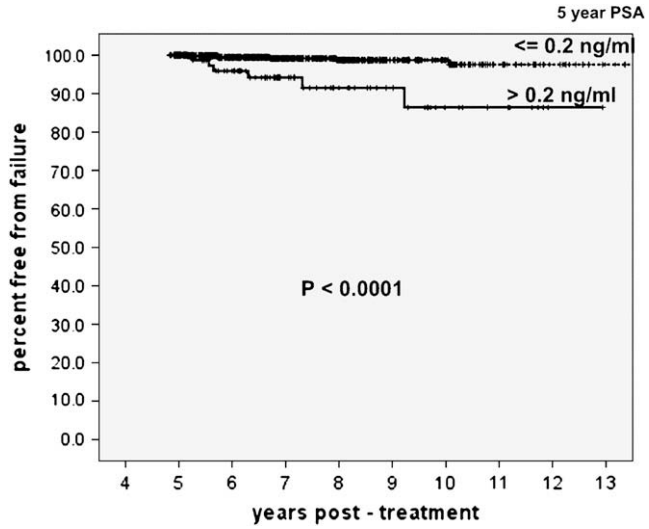

Fig. 3. Effect of 5-year prostate-specific antigen (PSA) level on biochemical failure using American Society for Therapeutic Radiology and Oncology (ASTRO) definition.

dose delivery should lead to decreased late-failure rates. Because improved brachytherapy techniques have led to improved dose delivery, we compared the outcomes of patients treated in the earlier years of this study with those of patients treated more recently.

Consistent with improved dose delivery, the mean BED value for patients treated in 1998 or earlier vs. 1999 or later was 177 Gy2 ($n = 383$) and 205 Gy2 ($n = 359$), respectively ($p < .0001$). The percentage of patients with a 5-year PSA value of ≤0.2 ng/mL for these two groups was 59% and 76%, respectively ($p < .0001$). The BED did not significantly affect FFPF. The 10-year FFPF rate using the ASTRO definition was 95% and 95.5% ($p = .8$) and using the Phoenix definition was 96% and 99% ($p = .1$) for the BED group ≤180 Gy2 and >180 Gy2, respectively. In addition, when patients were stratified by treatment regimen, the BED still did not

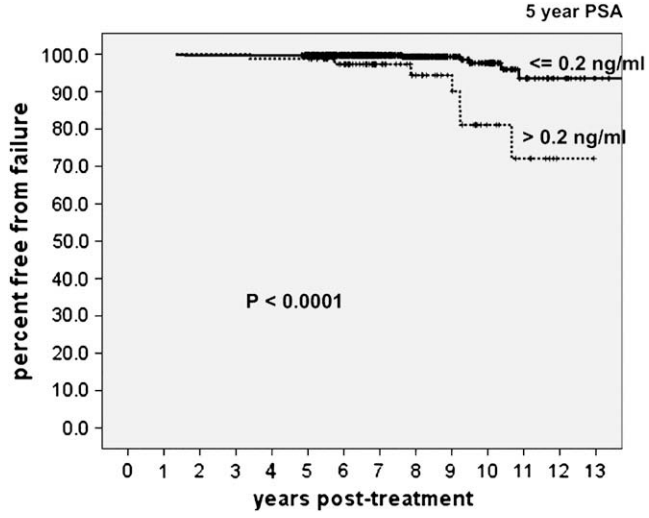

Fig. 4. Effect of 5-year prostate-specific antigen (PSA) level on biochemical failure using Phoenix definition.

Table 4. Effect of BED, by treatment group, on biochemical failure

Treatment group	BED (Gy2)	Patients (*n*)	10-y FFPF (%) ASTRO	10-y FFPF (%) Phoenix
BT alone				
	≤180	112	94	94
	>180	190	97.5	92
p			.2	.9
BT and HT				
	≤180	102	99	97
	>180	100	100	100
p			.3	.45
BT and EBRT (with or without HT)				
	≤180	18	100	100
	>180	201	99	98
p			.7	.8

Abbreviations: BT = brachytherapy; HT = hormonal therapy; EBRT = external beam radiotherapy; other abbreviations as in Table 2.

significantly effect FFPF (Table 4). Although the BED did not affect the FFPF rates, it did affect the mean 5-year PSA value, with a BED value of ≤180 Gy2 (n = 232) and >180 Gy2 (n = 491) corresponding to an average 5-year PSA value of 0.127 ng/mL and 0.082 ng/mL, respectively (p = .019).

DISCUSSION

Long-term outcomes and late failure

Previous studies of brachytherapy for prostate cancer have demonstrated excellent overall outcomes (13). The present data build on these studies, showing that patients who remain failure free in the first 5 years after prostate brachytherapy alone and combined with hormonal therapy and/or EBRT do not die of their prostate cancer at 10 years. In particular, the disease-specific survival and freedom from distant metastasis rates for our entire cohort were both 100%, with a 10-year FFPF rate of 95–97% depending on the criteria used. The low rate of biochemical failure after 5 years is similar to the findings of Shipley *et al.* (14), who noted that only 5% of patients who remained free of recurrence for 5 years developed subsequent failure from their fifth to eighth year after EBRT. In addition, in a previous report, patients followed up for a minimum of ≥5 years had excellent preservation of urinary, sexual, and rectal quality of life (15).

The issue of late failure and, in particular, late metastatic disease and their cause has been much debated, with arguments made for both the presence of microscopic metastatic disease at treatment and local persistence of tumor that metastasizes being responsible for late failure (16). If the first argument (micrometastasis) is correct, improved local control, whether with better dose delivery or enhanced surgical techniques, will have little or no impact on outcomes. Our data, however, support the second argument (local persistence). This has been demonstrated by the complete freedom from distant metastases in our cohort and the lower 5-year PSA values with improved dose delivery to the prostate. Patients with microscopic systemic disease tend to have treatment failure earlier than 5 years and local failures tend to recur latter. Although the numbers were small, the patients included in the present study who had positive nodes or positive seminal vesicle biopsy findings (prognostic factors for systemic disease) all did well with no evidence of biochemical failure. In general, most patients with this type of disease will develop treatment failure earlier than 5 years and thus would have been excluded from the present study.

Five-year PSA level as prognostic tool

Our data have demonstrated that the 5-year PSA value is a useful tool for predicting for FFPF for the subsequent 5 years of a patient's life. In particular, patients with a 5-year PSA value ≤0.2 ng/mL, which accounts for an increasing majority of our patients, will have a 1–2% risk of developing biochemical failure within the next 5 years, depending on the criteria used. Thus, the 5-year PSA value provides important information that should be used to update a patient's prognosis.

Defining cure

Investigators have shown that a PSA nadir is a prognostic factor predicting for biochemical recurrence, and some have suggested that it can be used as a surrogate for cancer control (17, 18). Our data suggest that the 5-year PSA level can be highly predictive of the 10-year cure rates. Patients with 5-year PSA values of ≤0.2 ng/mL were essentially free of subsequent failure. The decision to focus on the 5-year PSA value was supported by data from Critz *et al.* (19) who showed that 99% of patients who achieved a PSA level of <0.2 ng/mL after combined brachytherapy and EBRT did so by 5 years after treatment. Patients remaining free of biochemical failure during the first 5 years had an excellent prognosis for the 10-year period after treatment, with no patient developing distant metastases or dying of prostate cancer.

This finding is in marked contrast to the experience with retropubic prostate implantation of the late 1970s to mid-1980s that showed a propensity for late failure. Kuban *et al.* (20) showed that only 57% of recurrences with ^{125}I were clinically evident by 5 years of follow-up, with failures detected at ≤10 years. The main difference between their findings and the present analysis is that their data were from the pre-PSA era. In addition, the patients in the present study were also treated with brachytherapy combined with hormonal therapy and EBRT. With PSA measurement, most of these recurrences would have been detected earlier. In addition, because of the techniques used during the previous period, few implantations would have been deemed high quality as judged by today's standards. The inability to achieve good dose coverage most likely resulted in poor local control. Local failure is often detected late, especially without PSA measurement as a monitoring tool.

The present findings have implication for clinical practice and follow-up. Because patients whose 5-year PSA value is ≤0.2 ng/mL will rarely develop subsequent treatment failure over the next 5 years, they can be followed at less regular

intervals than the recommended every 6 months. These data support the National Cancer Care Network guidelines recommending annual follow-up visits after 5 years of follow-up. This would not only be more convenient for the patient, but would also reduce the overall cost of treatment.

Brachytherapy technique continues to improve

Our data have indicated that improvements are continuing and that these will continue to have an affect on prostate brachytherapy data for years to come. The BED values were significantly greater for the patients who were treated more recently in our cohort, correlating with both a decrease in the average 5-year PSA value and an increase in the percentage of patients who achieved a 5-year PSA value of <0.2 ng/mL. This suggests that the late failure rates will continue to decrease, making prostate brachytherapy alone and combined with hormonal therapy and/or EBRT an increasingly attractive treatment option.

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CLINICAL INVESTIGATION

RADIATION DOSE PREDICTS FOR BIOCHEMICAL CONTROL IN INTERMEDIATE-RISK PROSTATE CANCER PATIENTS TREATED WITH LOW-DOSE-RATE BRACHYTHERAPY

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Purpose: To evaluate the influence of patient- and treatment-related factors on freedom from biochemical failure (FFbF) in patients with intermediate-risk prostate cancer.

Methods and Materials: From a prospectively collected database of 2250 men treated at Mount Sinai Hospital from 1990 to 2004 with low-dose-rate brachytherapy for prostate cancer, 558 men with either one or more intermediate-risk features (prostate-specific antigen [PSA] level 10–20 ng/mL, Gleason score 7, or Stage T2b) were identified who had a minimum follow-up of 24 months and postimplant CT-based dosimetric analysis. Biologically effective dose (BED) values were calculated to compare doses from different isotopes and treatment regimens. Patients were treated with brachytherapy with or without hormone therapy and/or external-beam radiotherapy. Patient- and treatment-related factors were analyzed with respect to FFbF. The median follow-up was 60 months (range, 24–167 months). Biochemical failure was defined according to the Phoenix definition. Univariate analyses were used to determine whether any variable was predictive of FFbF. A two-sided p value of <0.05 was considered significant.

Results: Overall, the actuarial FFbF at 10 years was 86%. Dose (BED <150 Gy_2 vs. ≥150 Gy_2) was the only significant predictor of FFbF ($p < 0.001$). None of the other variables (PSA, external-beam radiotherapy, Gleason score, treatment type, hormones, stage, and number of risk factors) was found to be a statistically significant predictor of 10-year FFbF.

Conclusions: Radiation dose is an important predictor of FFbF in intermediate-risk prostate cancer. Treatment should continue to be individualized according to presenting disease characteristics until results from Radiation Therapy Oncology Group trial 0232 become available.

Prostate cancer, Brachytherapy, Intermediate risk.

INTRODUCTION

The optimal treatment of intermediate-risk prostatic adenocarcinoma is controversial. Radical surgery, brachytherapy, external-beam radiotherapy (EBRT), hormone suppression, and combinations of these modalities are all feasible treatment options (1). Different treatment strategies have been developed on the basis of pretreatment prognostic factors (2–5); however, retrospective studies have failed to demonstrate the superiority of one treatment regimen over another (6–9).

Over the past decade, low-dose-rate brachytherapy has emerged as a viable and commonly used treatment for clinically localized prostate cancer. Traditionally, brachytherapy alone has been used for low-risk prostate cancer (8). Most high-risk patients have been treated with combination therapy that includes brachytherapy, EBRT, and hormonal therapy (10, 11). However, the optimal method of incorporating brachytherapy into the management of intermediate-risk prostate cancer is debatable because there are few data to support the use of one regimen over the others in terms of local failure, biochemical (prostate-specific antigen [PSA]) failure, or overall survival (7, 12, 13).

Various brachytherapy regimens have been reported and include brachytherapy alone, combined hormonal therapy and implant, as well as brachytherapy in combination with EBRT (14–24). At Mount Sinai Hospital, various regimens for treating intermediate-risk patients have been used over a 14-year period. The purpose of the present study was to evaluate the influence of treatment-, tumor-, and patient-related factors on freedom from biochemical failure (FFbF) and to determine whether an optimal treatment approach exists for this particular risk group.

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METHODS AND MATERIALS

Patient selection

Between June 28, 1990, and December 1, 2004, a total of 2250 patients with T1–T3 prostate cancer were treated with low-dose-rate brachytherapy with or without androgen suppression and with or without EBRT at Mount Sinai Hospital. No patients had radiologic or pathologic evidence of metastatic disease. Of these patients, 626 were classified as intermediate-risk and had at least 24 months of follow-up. Of these 626 patients, 17 patients received EBRT alone and were therefore excluded from this analysis. Among the 609 remaining patients, postimplant dosimetry was unavailable for 51 patients, most commonly owing to patients failing to keep their postimplant dosimetry appointments. In total, 558 intermediate-risk patients had postimplant dosimetric information and form the study population for this article.

All patients were required to have biopsy-proven prostate cancer and were risk-stratified according to the Gleason sum determined at central pathologic review. The extent of disease workup included a thorough history and physical examination, followed by routine laboratory studies, pelvic CT, bone scan, and serum PSA determinations. Seminal vesicle (SV) biopsies, which were performed in 277 (49.6%) of the 558 patients, were done at the discretion of the treating urologist. In general, SV biopsy was used for patients with Gleason scores of 7, PSA $\geq$10 ng/mL, or clinical stage T2b; this resulted in the inclusion of 8 patients (2.9%) with biopsy-proven SV involvement. We included patients with positive results on SV biopsy in this study because although most intermediate-risk prostate cancer series did not evaluate the SV in this manner, these studies would have certainly included patients harboring SV disease. Of the 558 patients, 66 (11.8%) had lymph node dissections, all of which were negative for malignancy. Lymph node dissections were performed at the discretion of the treating urologist. Node-positive patients were not included in this analysis. All patients were staged according to the 1992 American Joint Committee on Cancer staging system (25).

Patient characteristics

The clinical stage, presenting Gleason score, presenting PSA value, SV invasion status, and the number of intermediate risk factors for all patients can be found in Table 1.

Table 1. Presenting clinical characteristics of men with intermediate-risk prostate cancer

Clinical stage	
≤T2a	297 (53)
T2b	261 (47)
Gleason score	
2–6	290 (52)
7	268 (48)
PSA (ng/mL)	
<10	322 (58)
10–20	236 (42)
No. of risk factors	
1	381 (68.3)
2	147 (26.3)
3	30 (5.4)
SV invasion	
Biopsy-proven	8 (2.9)
None	277 (49.6)
Unknown (not biopsied)	281 (50.4)

Abbreviations: PSA = prostate-specific antigen; SV = seminal vesicle. Values are number (percentage).

Intermediate-risk definition

Patients were defined as having intermediate-risk disease if their disease was characterized by one or more of the following characteristics: Stage T2b, Gleason 7, or initial PSA value 10–20 ng/mL (8).

Treatment: brachytherapy technique

All patients were implanted with use of a real-time transrectal ultrasound-guided technique. The original technique as described in 1995 and the impact of subsequent innovations have been previously reported (2, 26, 27). Iodine-125 (^{125}I) and palladium-103 (^{103}Pd) seeds were used. Patients with positive results on SV biopsy additionally underwent seed implantation into the walls of the vesicles, and all SV-positive patients received supplemental EBRT as outlined below. The activity range per seed was 0.3–0.5 mCi for ^{125}I and 1.0–1.5 mCi for ^{103}Pd. All patients received postimplant dosimetric analysis as described below.

Treatment: EBRT technique

Patients treated with EBRT received a median dose of 45 Gy (range, 39.6–61.2 Gy) delivered 6–8 weeks after the brachytherapy procedure. The median D90 (dose to 90% of the prostate volume, described in detail below) from partial-dose ^{103}Pd prostate seed implantation was 103.5 Gy (range, 57.2–144.4 Gy). In the earlier years of the study, lower implant doses were used with higher external-beam doses. In addition, the brachytherapist (R.G.S.) varied the EBRT prescription dose according to the dosimetric findings at the time of postimplant dosimetry. Between 1990 and June 2003, conformal three-dimensional irradiation was delivered with six fields (two anterior oblique, two posterior oblique, and two lateral). Starting in June 2003, intensity-modulated radiotherapy was delivered with five fields (two anterior oblique, two posterior oblique, and one posterior), utilizing a custom alpha cradle immobilization device. The target volume included the prostate and entire SV length, with a 15–20-mm margin to the block edge. Doses were typically prescribed to the isodose line that encompassed the entire prostate and SV, with a 5–15-mm margin. Position verification and correction were performed by using standard port film imaging during the three-dimensional conformal irradiation era and with orthogonal film isocenter verification during the intensity-modulated radiotherapy era.

Treatment: hormonal therapy

Hormonal therapy consisted of a gonadotropin-releasing hormone agonist (either leuprolide acetate or goserelin acetate) with or without an antiandrogen (either flutamide or bicalutamide). When hormonal therapy was combined with brachytherapy without EBRT, it was typically used for one of two reasons: for downsizing large prostate glands (gland size $\geq$50 cm^3), or as adjuvant therapy. In either case, it was usually given for 3 months before implantation and for 3 months after implantation (12).

Treatment: trimodality therapy

Trimodality therapy included 3 months of hormonal therapy followed by a ^{103}Pd brachytherapy implant (prescription dose, 100 Gy) and EBRT 4–6 weeks later to a dose of 45 Gy. The total duration of hormonal therapy in this case was 9 months. The presenting tumor characteristics by treatment type are exhibited in Table 2.

Table 2. Patients stratified by treatment and tumor characteristics

Factor	Implant	Implant + HRM	Implant + EBRT (±HRM)
Gleason score 2–6	15% (82)	25% (138)	13% (70)
Gleason score 7	2% (9)	11% (63)	35% (196)
PSA <10 ng/mL	9% (51)	19% (104)	30% (167)
PSA 10–20 ng/mL	7% (40)	17% (97)	18% (99)

Abbreviations: HRM = hormones; EBRT = external-beam radiotherapy; PSA = prostate-specific antigen.

Postimplant CT dosimetry and BED calculations

Patients underwent CT-based dosimetry 1 month after implantation. Computed tomography scans of the prostate gland were obtained using 3-mm abutting cuts. The dose delivered to the prostate was determined from a dose–volume histogram analysis and defined as the dose delivered to 90% of the prostate volume (D90). All doses were defined according to the American Association of Physicists in Medicine Radiation Therapy Committee Task Group 43 and National Institute of Standards and Technology 1999 guidelines (28, 29). To compare doses between different isotopes and between implant alone and combined implant and EBRT, biologically effective dose (BED) equations were used. Although the V100 and V90 (volume of the prostate receiving ≥100% and ≥90% of the prescription dose, respectively) values were available for each patient in this study population, the clinical relevance of the D90 dosing parameter has been previously described (30–32), and the D90 value has the advantage of being a parameter easily incorporated into BED calculations. The linear-quadratic model was used to determine the BED for EBRT treatments using the equation (33–35):

$$BED = nd(1 + [d/(\alpha/\beta)])$$

where n = number of fractions; d = dose per fraction; and α/β = a tissue- and effect-specific parameter associated with the linear-quadratic model. The equation used to calculate the BEDs for the low-dose-rate permanent decaying implants with ^{125}I and ^{103}Pd was:

$$BED = (R_0/\lambda)(1 + [R_0/\{(u + \lambda)(\alpha/\beta)\}])$$

where R_0 = initial dose rate of implant = (D90)(λ); λ = radioactivity decay constant = 0.693/$T_{1/2}$; $T_{1/2}$ = radioactive half-life of isotope; u = repair rate constant = 0.693/$t_{1/2}$; and $t_{1/2}$ = tissue repair half-time. The values used for these constants for prostate carcinoma were α/β = 2 Gy; $t_{1/2}$ = 1 h; $T_{1/2}$ = 60 days for ^{125}I and 17 days for ^{103}Pd (36–39). By convention, the unit of BED calculated when α/β = 2 Gy is Gy_2. The BED values for treatments including both implant and EBRT were calculated by adding the BEDs computed for each treatment (40). The relative contribution to BED values for typical EBRT doses and brachytherapy D90 values can be illustrated by the following two examples: (*1*) 45 Gy EBRT as delivered above is equal to 85.5 Gy_2, and a D90 value of 100 Gy from a partial Pd^{103} implant is equal to 112.2 Gy_2, for a total BED value of 197.7 Gy_2; (*2*) a full-dose I^{125} implant with a D90 of 160 Gy is equal to a BED value of 168.9 Gy_2. Further information regarding BED calculations and the rationale for selection of an α/β ratio of 2 Gy for prostate cancer have been previously described in detail (41).

Follow-up

All patients were asked to return every 6 months after completion of treatment. Follow-up time was calculated from completion of treatment to last available follow-up date or date of death and ranged for the entire population from 2 to 14 years (median, 5 years).

Treatment endpoints

Freedom from biochemical failure was used as a surrogate for disease-free survival. The Phoenix consensus definition of PSA failure, defined as the nadir PSA value plus 2 ng/mL without backdating, was used (42).

Statistics

Freedom from biochemical failure curves were calculated using the methods of Kaplan and Meier (43). Differences in FFbF rates were calculated using the log–rank test. A χ^2 analysis was used to compare differences between groups.

RESULTS

The overall FFbF rate at 10 years for all patients in this study was 86% (Fig. 1). A total of 46 patients failed treatment as defined by the Phoenix consensus definition. The median time to failure was 43 months.

On univariate analysis, various patient-, tumor-, and treatment-related characteristics were analyzed with respect to effect on 10-year FFbF. Of the eight factors tested (stage, hormonal therapy, number of risk factors, treatment type, BED, Gleason score, PSA level, and use of EBRT), only BED was found to be significant ($p < 0.001$). Because only one significant prognostic factor (BED) was identified on univariate analysis, multivariate analysis was not performed. Each factor and its respective p value can be found summarized in Table 3.

To analyze the effect of increasing BED on FFbF, patients were divided into five BED dose groups. The dose groups were ≤120 Gy_2 (n = 33), >120–140 Gy_2 (n = 24), >140–160 Gy_2 (n = 42), >160–180 Gy_2 (n = 82), and >200 Gy_2 (n = 377). Generally, patients who were treated during the early years of the study received the lower BED values.

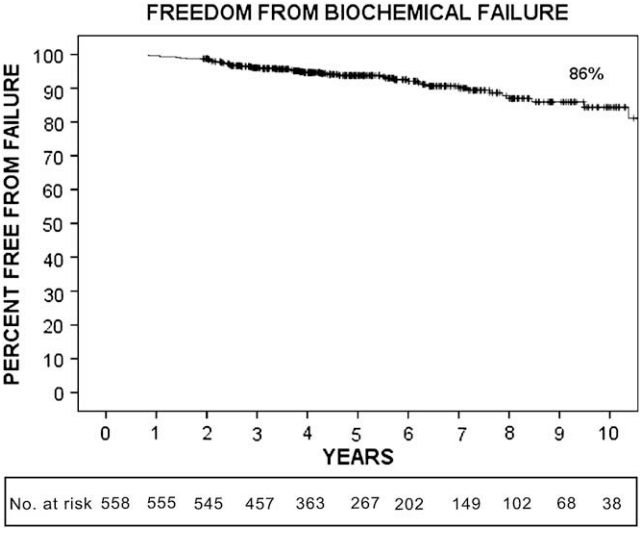

Fig. 1. Actuarial freedom from biochemical failure (FFbF) for all intermediate-risk patients in the study. The 10-year FFbF was 86% using the Phoenix definition.

Table 3. Univariate analysis of factors that may affect PSA failure

Factor	10-y FFbF (%)	*p*
Stage		0.18
≤T2a	92	
T2b	80	
HRM use		0.13
Yes	88	
No	78	
No. of risk factors		0.10
1	90	
2	73	
3	92	
Implant status		0.11
Implant alone	76	
Implant + HRM	89	
Implant + EBRT + HRM	91	
BED		<0.001
<150 Gy_2	63	
>150 Gy_2	92	
Gleason score		0.57
≤6	76	
7	80	
PSA		0.97
≤10 ng/mL	84	
10–20 ng/mL	84	
EBRT		0.77
Yes	91	
No	84	

Abbreviations: PSA = prostate-specific antigen; FFbF = freedom from biochemical failure (Phoenix definition); HRM = hormones; BED = biologically effective dose; EBRT = external-beam radiotherapy.

The median follow-up for the five groups was 9.77 years, 7.74 years, 8.05 years, 6.15 years, and 4.29 years, respectively. The 10-year FFbF rate for BED groups ≤120 Gy_2, >120–140 Gy_2, >140–160 Gy_2, >160–180 Gy_2, and >180 Gy_2 was 58%, 69%, 86%, 93%, and 92%, respectively ($p < 0.001$). This improvement in 10-year FFbF with increasing BED dose is displayed in Fig. 2 using a threshold BED value of 150 Gy_2, which is a dichotomization value that was previously shown to be associated with a significant difference in 10-year FFbF favoring higher dose (41).

In our overall population of 558 patients, the median BED in patients who did and did not receive EBRT in addition to implant was 207 Gy_2 and 176 Gy_2, respectively. A significantly greater proportion of patients with two or more intermediate risk factor received EBRT (114 of 177, or 65%) compared with patients who had only one intermediate risk factor (152 of 381, or 40%) ($p < 0.001$).

Of the 588 patients in our study population, 488 (87.4%) constituted a high-dose subset of patients (BED ≥150 Gy_2). The addition of EBRT in this select cohort did not significantly impact 10-year FFbF, with 10-year FFbF of 91% and 94% in those with and without EBRT, respectively ($p = 0.10$). These results are illustrated in Fig. 3.

Further analysis on the effect of treatment type and hormonal therapy in this subset of patients with a BED ≥150 Gy_2 was performed. The distributions of treatment regimens among this dose group were as follows: 11.6% (57) implant alone, 33.8% (165) implant and hormonal therapy, and 54.5% (266) implant and EBRT with or without hormonal therapy. There was no significant difference in outcome among the patients treated the various treatment regimens when the BED was greater than 150 Gy_2: the 10-year FFbF rate was 89%, 96%, and 91%, respectively ($p = 0.23$). These results are demonstrated in Fig. 4.

A significantly greater proportion (78%) of patients with BED ≥150 Gy_2 received hormonal therapy, compared with patients with BED <150 Gy_2 (50%) ($p < 0.001$). As demonstrated in Fig. 5, the addition of hormonal therapy in the high-dose (BED ≥150 Gy_2) subset was not found to significantly impact 10-year FFbF, with a rate of 93% for those receiving

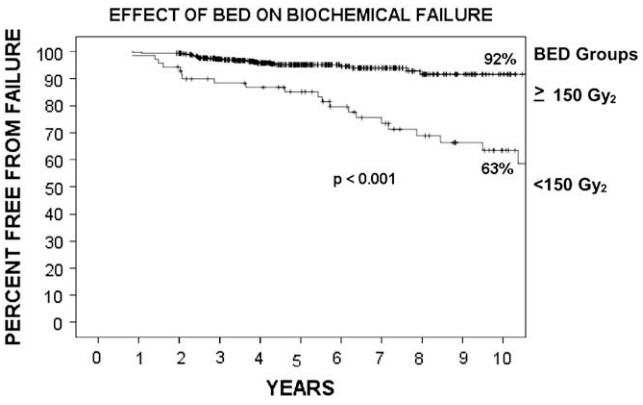

Fig. 2. Effect of biologically effective dose (BED) on freedom from biochemical failure (FFbF) in men treated for intermediate-risk prostate cancer. For patients with a BED ≥150 Gy_2, the 10-year FFbF was 92% using the Phoenix definition ($n = 488$ with 25 events), whereas for patients with a BED <150 Gy_2, the 10-year FFbF was 63% ($n = 70$ with 21 events) ($p < 0.001$).

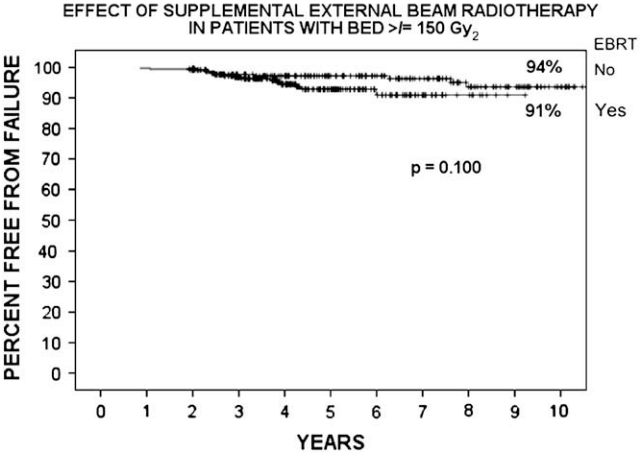

Fig. 3. Effect of supplemental external-beam radiotherapy (EBRT) on freedom from biochemical failure (FFbF) in men treated for intermediate-risk prostate cancer with biologically effective doses (BEDs) of ≥150 Gy_2. For men treated without supplemental EBRT, 10-year FFbF was 94% using the Phoenix definition ($n = 223$ with 10 events), whereas for men treated with supplemental EBRT, 10-year FFbF was 91% ($n = 265$ with 15 events) ($p = 0.100$).

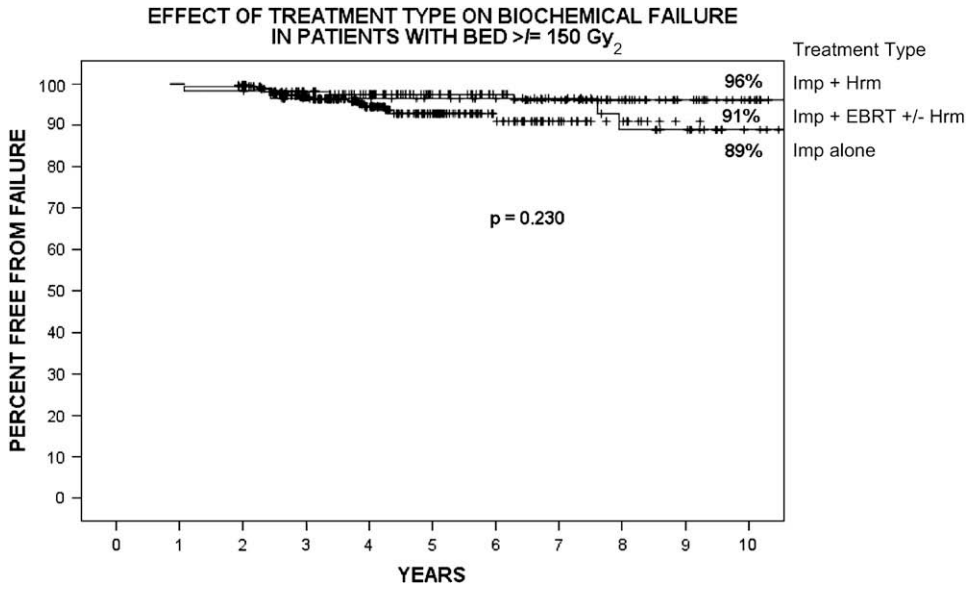

Fig. 4. Effect of treatment type (implant [Imp] alone, implant plus hormones [Hrm], and implant plus external beam radiotherapy [EBRT] with or without hormones) on freedom from biochemical failure (FFbF) in men treated for intermediate-risk prostate cancer with biologically effective doses (BEDs) of ≥150 Gy_2. For men treated with implant and hormones, the 10-year FFbF was 96% using the Phoenix definition (n = 165 with 6 events); for men treated with implant plus EBRT with or without hormonal therapy, 10-year FFbF was 91% (n = 266 with 15 events); for men treated with implant alone, 10-year FFbF was 89% (n = 57 with 4 events) (p = 0.230).

hormonal therapy vs. 88% for those treated without hormonal therapy (p = 0.88).

DISCUSSION

Overall, implant alone, implant plus hormones, and trimodality therapy all represent effective therapeutic options for intermediate-risk prostate cancer patients. These results confirm the findings of multiple retrospective trials, which established the equivalence of each in terms of biochemical relapse-free survival. A recently published prospective series of 300 men demonstrated 5-year actuarial FFbF of 93% in 111 intermediate-risk patients with the use of brachytherapy alone (23). In our study, the 10-year FFbF was 92% in men whose BED was ≥150 Gy_2, compared with 63% in men with BED <150 Gy_2.

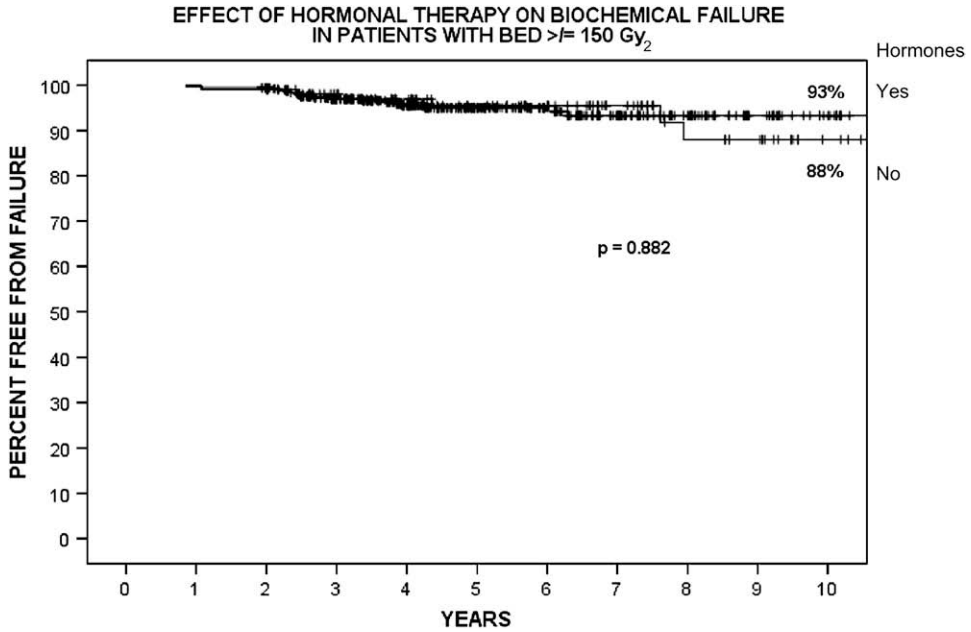

Fig. 5. Effect of hormonal therapy on freedom from biochemical failure (FFbF) in men treated for intermediate-risk prostate cancer with biologically effective doses (BEDs) of ≥150 Gy_2. For men treated with hormonal therapy, 10-year FFbF was 93% using the Phoenix definition (n = 381 with 19 events), whereas for men treated without hormonal therapy, 10-year FFbF was 88% (n = 107 with 6 events) (p = 0.882).

Biologically effective dose is clearly an important treatment variable in achieving optimal FFbF. Our data show that a high BED can be achieved with either the combination of brachytherapy and EBRT or with a high-quality brachytherapy implant alone. A BED cutpoint at which dose no longer impacts biochemical control could not be identified. Biologically effective dose seems to continuously impact FFbF rates, as demonstrated by the positive correlation between increasing BED groups and FFbF rates. Radiation dose seems to matter in all patients, regardless of risk stratification. Intermediate-risk patients may, in fact, require doses even higher than those that have typically been prescribed in other series. At our institution, the prescription dose for a monotherapy ^{125}I implant is 160 Gy, whereas the dose for a partial ^{103}Pd implant is 100 Gy plus 45 Gy EBRT. These doses correspond to BED values of 169 Gy_2 and 198 Gy_2, respectively. Thus, BED values >150 Gy_2 are achievable with ^{125}I implant alone or with combined-modality therapy.

The 96% 10-year FFbF rate achieved in our patients who received high-dose implants (BED $\geq$150 Gy_2) plus hormone therapy may support the argument that EBRT may be safely omitted in this particular subset of patients. However, this study was not designed to answer the question of whether the addition of EBRT to implant suffices in the treatment of intermediate-risk prostate cancer patients, because the majority of our patients who received EBRT also received hormonal therapy (216 of 266, or 81%).

The addition of hormone therapy demonstrated a trend toward improved PSA control (88% vs. 78%), although this difference was not statistically significant (p = 0.13). This effect was seen both in the overall patient population as well as in the high-dose subset, thereby highlighting the potential role of hormonal therapy in intermediate-risk patients. These data lend support to our previous finding that adding hormones to seed implantation may be of benefit in men with intermediate-risk disease (12).

Comparisons of treatment modalities in intermediate-risk patients have been hindered by various definitions of *intermediate risk* by different investigators. In the present study, the presence of two or more intermediate risk factors did not impart a worse prognosis than the possession of only one intermediate risk feature, thereby implying that these patients, who are typically categorized as high risk, should still be eligible for implant as a component of their therapy.

Several aspects of our study may be viewed as potential limitations. Given the retrospective nature of our study, selection bias was present, and treatments were individually tailored according to presenting disease characteristics. In general, combined-modality therapy was recommended to patients with more aggressive tumor features, such as a high percentage of involved core biopsy specimens or the presence of two or more intermediate risk factors. Because it is not routinely reported, we were unable to quantify, capture, and analyze the impact of percentage of pathologically involved cores on PSA failure.

Intermediate-risk prostate cancer patients represent a heterogeneous group with various presenting tumor characteristics. Therefore, customizing treatment options on a case-by-case basis is necessary. Selection criteria for the group of intermediate-risk patients who may benefit from combined implant and EBRT have yet to be defined. These issues highlight the importance of enrolling patients on Radiation Therapy Oncology Group (RTOG) trial 0232, which is an active Phase III study designed to compare combined EBRT and prostate brachytherapy with brachytherapy alone in intermediate-risk prostate cancer.

CONCLUSION

In the absence of evidence from randomized controlled trials, implant alone, implant plus hormones, and trimodality therapy all seem to represent effective therapeutic options for patients with intermediate-risk prostate cancer. Radiation dose is an important predictor of biochemical control in this group of patients. This observation, however, requires prospective validation, and we await the results of the currently accruing RTOG 0232 trial, which hopefully will illuminate the optimal treatment plan for this select patient population.

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Stereotactic Radiosurgery for Thoracic Malignancies

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Radiosurgery for lung cancer is a novel and promising concept that warrants thorough review. Stereotactic body radiotherapy enables the selective delivery of an intense dose of high-energy radiation to destroy a tumor with precise targeting. The radiobiology and physics behind the use of radiosurgery are presented, followed by a discussion of promising retrospective and prospective clinical data that has been reported from Japan, Europe, and the United States. The article closes with a discussion of multidisciplinary approaches that include radiosurgery which are on the therapeutic horizon.

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Radiosurgery is the application of very high doses of ionizing radiation in larger than traditional fractionation to much smaller than traditional radiotherapy fields, often with the integration of advanced modalities for tumor imaging and devices for tumor immobilization. The concept of radiosurgery was conceived in the early 1950s by a Swedish neurosurgeon, Lars Leskell, and its application in the brain and spine have been consistently applied for both malignant and benign conditions ever since [1, 2]. Body radiosurgery for lung cancer is an offshoot of radiosurgery for the brain and spine. Much is known about the doses used for effective control of brain tumors and cerebral metastatic deposits, and the lung radiosurgery literature is informed by these experiences [3, 4].

In this article, we will discuss such issues as the definition of lung radiosurgery, the radiobiology of large radiation doses, considerations of tumor targeting, and reported efficacy and toxicity of the approach. It is important to remind the reader that the rapidly evolving literature for lung radiosurgery has occurred within the context of the long-standing successful clinical outcomes of this approach in the neurosurgical realm, and considering the many decades it has taken to fully adopt this brain technology, it is safe to conclude that the clinical application of radiosurgery to the lung remains in its early investigational stages.

Definition

The definition of radiosurgery for brain lesions had been much pondered, although to our knowledge, no such effort has been made for lung radiosurgery. A review of the available published reports of authors claiming to have performed lung radiosurgery allows for a deduction of an inclusive definition. Radiosurgery for thoracic lesions appears at its simplest to require higher than 3-Gy fractions, which combined add up in biologic effectiveness to a total dose in 2-Gy equivalents that exceeds the 66 to 74 Gy that can be given safely with modern three-dimensional treatment planning. This technique uses fewer than traditional treatment fractions (approximately 10 or less) with the integration of either respiratory gating or attempted tumor immobilization, resulted in a marked reduction in the amount of normal tissue exposed to the therapeutic dose.

Lung radiosurgical efforts at this point are represented by a spectrum of technology. Many of the largest reported series have used standard linear accelerators with usually a pioneer's eye on immobilization, tumor identification, and respiratory motion [5–7]. There are now several advanced body radiosurgical treatment devices that appear to offer the possibility for institutions to replicate the pioneering efforts of others with a potentially more favorable therapeutic ratio [8–12] (Figure 1).

Radiobiology

Radiobiology is considered one of the three central pillars of radiation oncology. Much is known about the response of cancer and normal tissue to fractionated therapeutic radiation. The use of large fraction sizes in radiosurgery may not take advantage of one of the most valuable attributes associated with the use of small fraction sizes, which is its ability to spare normal tissue while curing the cancer. It is, however, important to note in this context that Fuks and Kolesnick [13, 14] have

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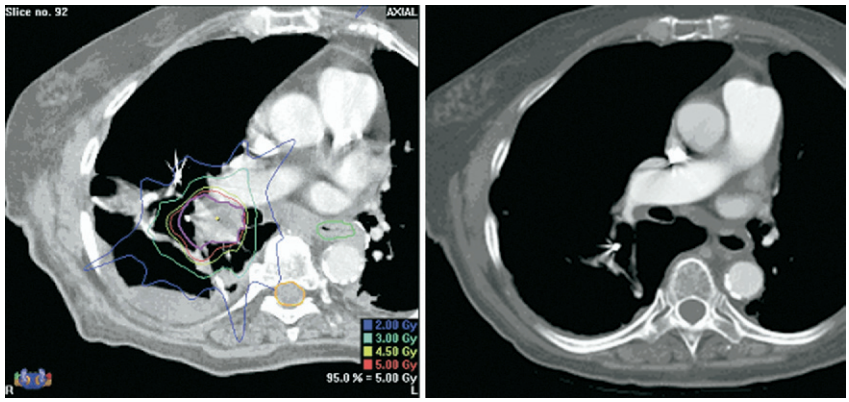

Fig 1. These computed tomography scans (CT) are of an 80-year-old female ex-smoker with a history of chronic obstructive pulmonary disease and a prior T2 N1 non-small cell lung cancer of the left lower lobe for which she received a left lower lobe sleeve resection and mediastinal chemoradiotherapy to 50.4 Gy in 1999. She presented in 2007 with another T2 N1 tumor now of the right lower lobe with compromised respiratory function. She underwent a wedge resection of the primary tumor and a course of reirradiation to the N1 lymph node agglomeration. (A) Represents the radiotherapy plan using the Novalis radiosurgical system (BrainLAB AG, Feldkirchen, Germany). (B) This CT scan 1 month after delivery of 50 Gy in 10 fractions reveals a complete radiologic response to the therapy.

proposed that the use of large fraction sizes greater than 8 to 10 Gy may be advantageous for tumor control due to the ability of large doses to induce endothelial cell damage, thereby causing microvascular dysfunction that enhances the killing of tumor cells. Evidence has been obtained that large radiation doses induce the translocation of endothelial cell acid sphingomyelinase into the plasma membrane of these cells where it is hydrolyzed to sphingomyelin. This results in the generation of ceramide, which is a second messenger that stimulates apoptosis of the endothelial cells [13–15].

In the thorax, the use of standard fraction sizes has allowed for a significant therapeutic gain in some settings and shortcomings in others. The effect of large fraction sizes can be quantified in its efficacy relative to the use of standard 1.8- to 2.0-Gy fractions through calculation of the biologically effective dose (BED) [16]. The equation commonly used is: $BED = nd[1 + (d/\alpha/\beta)]$ where n is the number of fractions; d is the dose per fraction; and α/β is a specific measured parameter unique to the tissue or cancer in question. Of interest, when referring to α/β for lung tumors is that a value of 10 Gy is often used, whereas the α/β for lung fibrosis is approximately 3 Gy. It should be noted that the BED to the tumor with radiosurgery is much higher than values with conventional radiation techniques, hence the better local control rates demonstrated in radiosurgery series.

Table 1. Biologically Effective Doses for Various Fractionated Radiation Regimens

Total Dose (Gy)	Dose/Fraction (Gy)	BED (Gy_{10})[a]
54	1.5	62.1
70.2	1.8	82.8
60	3.0	78.0
66	3.0	85.8
50	5.0	75.0
15	15	37.5[b]
30	30	120.0[b]

[a] BED values were calculated assuming an α/β ratio of 10 Gy. [b] The calculated BED values may not be valid for a protocol involving only one fraction.

BED = biologically effective doses.

The BED allows one to compare different radiation dose schedules across series and allows for a rational basis for dose escalation and alterations from standard protracted radiation protocols (Table 1). The high doses afforded by radiosurgery allow for the delivery of high BED with the convenience of a much shorter treatment time. In addition, there is no interval between radiation fractions, which means that the tumor is not allowed to grow between fractions.

There are theoretical downsides of radiosurgery that are worth noting. A reason tumor control may be diminished through the use of a small number of large fractions is that the ability for tumor cells to reassort into more sensitive phases of the cell cycle during the interval between fractions may be limited. Also, reoxygenation, a potentially radiosensitizing effect of fractionated radiation, is reduced as a result of the use of a small number of fractions [17].

Of interest is that the dose-rate of radiation therapy may also be a significant issue associated with the use of stereotactic radiosurgery because the rate at which the radiation is delivered can be lower than for a standard radiation treatment. This may allow one to differentiate between the various radiosurgical devices [18]. Repair during the course of the irradiation would generally be considered negligible for dose-rates greater than about 2 Gy/min. If irradiations are delivered at lower dose rates, however, and the repair that occurs during the irradiation may be significant, thereby diminishing the effectiveness of the dose delivered. To our knowledge, this effect has not been well quantified among the various lung radiosurgery techniques or quantified in terms of a

Table 2. Available Body Radiosurgical Systems, Manufacturer, and Marketing Claims

Name	Manufacturer	Marketing Claim[a]	Web site address
Axesse	Elekta AB	"An image-guided robotic linear accelerator that combines high-conformance beam shaping with our exclusive 4D Adaptive image guided radiation therapy (IGRT) technology for advanced stereotactic radiation treatments."	www.elekta.com
Cyberknife	Accuray Inc	"The world's first and only commercially available radiosurgery system designed to treat tumors anywhere in the body with sub-millimeter accuracy."	www.accuray.com
Hi·Art	TomoTherapy Inc	"Revolutionary design. Complete integration. Unparalleled precision. True accuracy. Full assurance."	www.tomotherapy.com
Novalis	BrainLAB AG	"Achieves consistent, superior dose distribution, for a larger range of indications, in less time and with high precision."	www.novalis-surgery.com
Primatom	Siemens AG	"This technology brings image guidance to radiation treatment delivery, providing accurate, near real-time target localization within the treatment room."	www.usa.siemens.com
Trilogy	Varian Medical Systems Inc	"The world's first image-guided radiation therapy system optimized for both conventional and stereotactic approaches to treating cancer."	www.varian.com
XKnife	Integra Radionics Inc	"The Body System is a non-invasive relocatable device that provides immobilization and localization for any affected body area."	www.radionics.com

[a] From the manufacturers' promotional material.

time-corrected BED for each available radiosurgical device.

The dose range used for radiosurgery of the lung ranges from single fractions as large as 30 Gy to multiple fractions of 4 to 5 Gy [9, 19]. It has been found that a BED of greater than 100 Gy_{10} given radiosurgically offers improved outcomes compared with treatment at a lower BED [20, 21]. This serves as a basis for comparison of more traditionally fractionated radiotherapy protocols that have a BED ranging from 60 to 90 Gy_{10} using an α/β of 10 Gy for lung cancer. Theoretically, based on dose-equivalency studies using the BED, one should be able to give either a few large fractions or multiple fractions and compare and roughly predict the outcome for the different regimens based on the applicable BED benchmark. This may offer a flexible approach toward dosing in prospective trials, because in lesions that are close to radiosensitive structures, a high BED can presumably be achieved with a more protracted radiosurgical approach. This would lessen the incidence of severe toxicity, whereas a lesion in the periphery of the lung could be treated with one fraction. This would allow accrual of both patients with central or peripheral types of tumor to the same radiosurgical study and allow the clinician the freedom to make modifications determined by the assessment of potential late effect risk unique to the patient.

Physics

There are currently at least seven devices capable of body radiosurgery (Table 2), several of which have been used for lung radiosurgery and are reported in the literature [9–12, 22]. They differ in important ways regarding their shielding requirements, vault size requirements, and physics staff support requirements. They are similar in that they initially require a large outlay of capital funds and a devoted oncology team to assure use of the device is appropriate to the medical needs of the institution and community. To date, no studies have directly compared the efficacy of these stereotactic radiosurgical devices in the treatment of lung cancer.

Tumor Immobilization

At present, and as will be discussed below, a major indication for lung radiosurgery in the United States and Europe is for the patient who is medically inoperable with a potentially curable cancer. It is reasonable to generalize that these patients as a group are extremely compromised in terms of cardiopulmonary status. The technology for respiratory "gating" has been reported, although its practical use in patients who are candidates for lung radiosurgery is extremely problematic. It is therefore difficult to make a recommendation that radiosurgery in the lung is predicated on the use of respiratory gating. Many have attempted successfully to limit diaphragmatic movement by abdominal pressure devices or to train the patient to limit the volume of their respiration for short periods [6, 7, 9, 23]. These approaches have been found to be both practical and workable; accuracy in targeting to less than a centimeter is an admirable theoretic possibility and practical in a comparatively few patients. Therefore, we recommend a practical approach at this point, which would include the addition of a patient-specific planning target volume quantified under direct observation using fluoroscopy [6, 24]. Interventions such as trained breathing or abdominal compression

Table 3. Outcome of Fractionated Radiotherapy in Addition to Selected Outcomes of Lung Radiosurgery

First Author	Patient No.	Stage I/II	Total dose/fractionation	Local Control[a]	End Point
Saunders [25]	338	36%	54 Gy/1.5 Gy TID from CHART phase III randomized trial	17%	3 years actuarial
Rosenzweig [26]	104	28%	70.2 Gy, 75.6 Gy, 81 Gy, 84 Gy, 90 Gy at 1.8 Gy/d from phase I trial	52%	2 years actuarial
Bradley [27]	56	100% (stage I)	Median isocenter dose 70 Gy (range, 59.94–83.85) at 1.8 Gy or 2 Gy/d single-institution experience	63%	3 years actuarial
Onishi [20]	245	100% (stage I)	18–75 Gy at isocenter in 1–22 fractions	86.5% local (91.8% regional) 73.6% if BED $<$ 100 Gy 91.95 if BED $\geq$ 100 Gy	Median follow-up, 24 mon
Timmerman [23]	70	100% (stage I)	60–66 Gy in 20–22 Gy fractions	95%	2 years actuarial
Le [22]	32	100% (stage I)	15–30 Gy as a single fraction	91% $>$ 20 Gy 54% $<$ 20 Gy	1 year actuarial

[a] Local control defined as no evidence of progression or recurrence at the treated site within the lobe.

BED = biologically effective dose; CHART = continuous hyperfractionated accelerated radiotherapy; TID = three times daily.

should be encouraged as only an improvement in the therapeutic ratio would result. At the University of Pittsburgh, the initial experience with stereotactic radiosurgery utilized a breath hold technique [19]. Currently, a dynamic tracking system (Cyberknife; Accuray, Sunnyvale, CA) for tracking the tumor during breathing is used.

Clinical Results

The preliminary results of stereotactic radiosurgery (SRS) for the lung have been encouraging, and it appears that local control is far superior to more traditional methods of radiation (Table 3) [20, 22, 23, 25–27]. One of the most influential studies of SRS for lung cancer is a multicenter trial from Japan of 245 patients with stage I non-small cell lung cancer (NSCLC) who were treated with SRS [20]. In contrast to other trials that have primarily included medically inoperable patients with potentially resectable cancers, this study included 87 patients who were considered good-operative-risk candidates. Overall survival was estimated to be 56% at a median follow-up of 24 months, and 47% at 3 years and 5 years. In the 87 patients who were operative candidates, the estimated 5-year survival was 88% in patients who received more than 100 Gy (BED). In the good-operable-risk patients who received less than 100 Gy (BED), survival was 69%. The higher dose of 100 Gy (BED) appears to be important to achieve better local control. In the whole cohort of 245 patients, local progression was 26.4% in the patients who received less than 100 Gy and was 8.1% in patients who received more than 100 Gy.

Given the decades-long experience of thoracic surgeons who have performed innumerable lobar and sublobar resections for lung cancer, these results appear to be almost too good to be true. This has lead to the premature suggestion that it may now be appropriate to perform a large randomized trial of SRS and lobectomy in good-risk patients with NSCLC [28]. It is true that these results appear to be encouraging, but we have several reservations about the initiation of such a trial.

- In the Japanese trial by Onishi and colleagues, local recurrence was only scored if a recurrence occurred at the tumor. In most surgical studies, local recurrence has included, at a minimum, a recurrence anywhere within the same lobe or the associated hilar lymph nodes, or both [29].
- Computed tomography scanning has the significant shortcoming of frequently underestimating the presence of nodal disease in situ and can therefore underestimate the frequency of disease recurrence within the associated lymph nodes after extremely conformal radiosurgical treatment [30].
- Also unlike a lobectomy, the lymph nodes are evaluated by either a formal lymph node dissection or extensive sampling procedure, allowing for radiologically occult disease to be identified and for the patient to be properly risk-stratified regarding the possible efficacy of adjuvant chemotherapy or mediastinal radiotherapy, or both.
- In addition, if regional nodal disease had been included in the definition of local failure in the Onishi study, local recurrence would have been 36.5% for tumors treated with less than 100 Gy and 15% for those tumors treated at more than 100 Gy, numbers much more in line with what one would expect from such a conformal approach to only the tumor and immediately adjacent tissues [20].
- The locoregional recurrence rate of 15% is more similar to the recurrence rates seen after wedge resection, although the reported follow-up duration after wedge resection is longer.

Therefore, this very optimistic series should not serve as basis for proceeding with a randomized trial of lobec-

tomy vs radiosurgery. Far too little is yet known about optimal patient selection, optimal radiation dose, necessary preprocedural diagnostic maneuvers, optimal radiation treatment volume, and follow-up imaging interpretation to invest the resources necessary for such a trial with so many serious unknowns remaining to be defined.

Another issue that should also be considered is that there appears to be differences in outcome for both radiosurgery and resection between Japanese and North American reports [31, 32]. This may be related in part to differences in tumor biology between tumors seen in the United States and Europe vs those seen in Japan. Among the 245 patients reported in the Japanese trial, 109 had adenocarcinomas and 26 had other histologies that were not defined. The specific number of bronchiolar cancers, which would have had a more indolent biology, was not reported.

Stereotactic radiosurgery really has not been studied in a population of good-surgical-risk patients outside of Japan; therefore, results outside of Japan have not been as potentially encouraging. Timmerman and colleagues [7] reported their initial experience with SRS in 37 high-risk patients with NSCLC. Tumor response was seen in 87% of these patients, with 27% demonstrating a complete response. At a median follow-up of 15 months, 6 patients experienced local failure. The median time to local progression was 13 months. The disease-free survival was 50%, and overall survival at a median follow-up of 15 months was 64%. This study did meet its goal of demonstrating that it is feasible to deliver high doses of radiation to medically inoperable patients with NSCLC.

The same group of investigators later published a phase I dose-escalation trial in 47 patients [33]. Patients were initially treated with 8 Gy/fraction for 3 fractions (total, 24 Gy). The planned target dose for the study was 24 Gy/fraction over 3 fractions for a total of 72 Gy. The maximum tolerated dose (MTD) was achieved for T2 tumors in this series but not for the T1 tumors. Owing to excessive toxicity for the T2 tumors at the 72 Gy dose level, 66 Gy in three 22-Gy fractions was defined as the MTD for T2 tumors. For the entire group, local failure was seen in 10 of 47 patients (21.3%) at a mean of 15.7 months. Most (90%) of the local failures occurred at doses of less than 16 Gy/fraction (total dose of $<$ 48 Gy). Regional failure occurred in 10 patients (21.3%), but in only 4 patients (40%) treated at low radiation doses of less than 16 Gy. It is not surprising that local control was better with the higher radiation doses. This is a common phenomenon in the radiotherapy of unresected tumors [26, 27]. Regional recurrence, on the other hand, appears not to be affected by higher radiation doses and may be related to occult disease within the lung/thoracic cavity that is not treated at the time of SRS.

The same group then published their phase II trial results, using the MTD of 60 Gy in 3 fractions for T1 NSCLC and 66 Gy for T2 NSCLC that were defined in the above study [23]. The study cohort included 70 patients with stage I NSCLC who were followed up for a median of 17.5 months. At 3 months, 60% of patients demonstrated both a complete or partial response and 40% had stable disease, indicating that local control was initially excellent. At follow-up, 3 patients (4.2%) demonstrated local recurrence and disseminated disease was seen in 7 (10%). The estimated overall 2-year survival was 54%.

Of note, significant toxicity was appreciated in this trial using the 3-fraction approach. There were six deaths (8.5%) related to grade 5 toxicity directly attributable to the radiosurgical intervention. The 2-year freedom from toxicity in central tumors was 54%, significantly (p = 0.004) worse compared with 83% in peripheral tumors. This important finding suggests that in the future, a more protracted fractionation scheme should be pursued for central tumors. By prolonging fractionation to 5 or 10 sessions, the normal tissues of the hilum and mediastinum can be given time to recover and repair the DNA damage caused by each radiosurgical session, thereby allowing the normal tissue to recover.

Investigators from Stanford reported another dose-escalation study [22]. In this study, 32 patients with pulmonary tumors were treated with a single fraction ranging from 20 to 30 Gy using the Cyberknife radiosurgical device. Radiation-related complications occurred in 8 patients who had been at doses exceeding 20 Gy. Most of these toxicities (5 of 8) occurred in patients with central tumors at 5 to 6 months after therapy. The treatment-related mortality rate was significant, with three deaths (9.3%) attributed to radiation complications. As in the previous studies, higher radiation doses were associated with better local control. In patients treated with more than 20 Gy, 91% of patients demonstrated freedom from local progression at 18 months. In patients treated with less than 20 Gy, 54% demonstrated freedom from local progression. These authors reported a 1-year survival of 85% for stage I NSCLC patients.

These studies show that although local control with SRS can be excellent with higher doses, caution must be exercised in selecting the dose schema, particularly in patients with central lesions. It is important to balance the efficacy of the intervention with toxicity when SRS is used to treat patients with lung cancer.

The thoracic oncology group from Pittsburgh reported their initial experience in 32 patients with lung neoplasm treated with a median dose of 20 Gy in a single fraction [19]. More recently, they reported their experiences in 21 patients with no more than stage I NSCLC treated with SRS; 20 Gy in a single fraction was used most patients using the Cyberknife system [34]. There were no procedure-related deaths. At a median follow-up of 21 months, local progression occurred in 9 patients (42%), the median time to local progression was 12.3 months, and the median survival was 26.4 months (confidence interval 95%, 13.6-NR).

A Future Direction

Another alternative therapy to SRS that is increasingly being used for medically inoperable patients with NSCLC is radiofrequency ablation (RFA) [35, 36]. Radio-

frequency ablation is discussed in more detail elsewhere in this supplement. Currently, RFA has been demonstrated to be feasible and short-term results are encouraging. As with SRS, long-term outcomes are still needed. Although RFA and SRS can be regarded as competing therapies, there may be a role for the combination of these modalities [37]. Hypoxic cells, such as those in the center of a tumor, tend to be more resistant to radiation. Radiofrequency ablation tends to be more effective in these dense central areas of lung tumors and less effective in the more aerated lung surrounding a tumor. Radiofrequency ablation also results in reactive neovascularization of tissue at the periphery of the tumor, making the periphery more susceptible to radiation. This potential synergy of radiation and RFA has been investigated in a rat tumor model [38]. Animals treated with RFA or radiation demonstrated similar survival, and those that received both therapies demonstrated superior survival to either therapy alone.

The combination of RFA and radiation has been reported in humans. In one study, 24 medically inoperable patients with stage I NSCLC received RFA and external beam radiation to a dose of 66 Gy [37]. At a mean follow-up of 26.7 months, there were 14 deaths (58.3%), of which 10 (41.7%) died of cancer, and three (12.5%) of respiratory failure. Although not specified in the article, it is possible that the protracted course of external beam radiation used in these high-risk patients may have added potentially avoidable toxicity to the lungs. We suggest that the alternative radiation approach of radiosurgery rather than a less conformal technology be used in combination with RFA. This combination is likely to be better in terms of tumor control and the minimization of potential morbidity risk.

Conclusion

In conclusion, SRS is feasible for NSCLC and appears to be superior to heavily fractionated external beam radiation when used as primary therapy for early-stage NSCLC. A number of issues remain to be resolved, including determining which version of the current conceptions of SRS is optimal for NSCLC in terms of safety and tumor control. Although higher radiation doses allow better local control, morbidity and mortality are increased if the dose is given as a single fraction or as a few fractions, particularly for central tumors. In some SRS series, procedure-associated mortality was much higher with high SRS doses than would be acceptable even after resection, RFA, or a sublobar resection for NSCLC.

Therefore, until further data is available, SRS for NSCLC should be done in the setting of a multidisciplinary thoracic oncology team and reserved for the high-risk patient. In addition, considering the promising results of SRS and RFA as monotherapy, we believe that a multimodality approach rationally combining both procedures offers the potential to further improve the therapeutic ratio in favor of oncologic intervention for the subset of medically inoperable patients with potentially curable tumors.

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CLINICAL INVESTIGATION **Prostate**

TGFB1 SINGLE NUCLEOTIDE POLYMORPHISMS ARE ASSOCIATED WITH ADVERSE QUALITY OF LIFE IN PROSTATE CANCER PATIENTS TREATED WITH RADIOTHERAPY

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Purpose: To investigate whether the presence of single nucleotide polymorphisms (SNPs) located within *TGFB1* might be predictive for the development of adverse quality-of-life outcomes in prostate cancer patients treated with radiotherapy.

Methods and Materials: A total of 141 prostate cancer patients treated with radiotherapy were screened for SNPs in *TGFB1* using DNA sequencing. Three quality-of-life outcomes were investigated: (*1*) prospective decline in erectile function, (*2*) urinary quality of life, and (*3*) rectal bleeding. Median follow-up was 51.3 months (range, 12–138 months; SD, 24.4 months).

Results: Those patients who possessed either the T/T genotype at position −509, the C/C genotype at position 869 (pro/pro, codon 10) or the G/C genotype at position 915 (arg/pro, codon 25) were significantly associated with the development of a decline in erectile function compared with those who did not have these genotypes: 56% (9 of 16) vs. 24% (11 of 45) ($p = 0.02$). In addition, patients with the −509 T/T genotype had a significantly increased risk of developing late rectal bleeding compared with those who had either the C/T or C/C genotype at this position: 55% (6 of 11) vs. 26% (34 of 130) ($p = 0.05$).

Conclusions: Possession of certain *TGFB1* genotypes is associated with the development of both erectile dysfunction and late rectal bleeding in patients treated with radiotherapy for prostate cancer. Therefore, identification of patients harboring these genotypes may represent a means to predict which men are most likely to suffer from poor quality-of-life outcomes after radiotherapy for prostate cancer. © 2008 Elsevier Inc.

Transforming growth factor β1 (TGF-β1), Single nucleotide polymorphisms (SNPs), Prostate cancer, Radiotherapy, Radiogenomics.

INTRODUCTION

The potential ability to predict both normal tissue and tumor response to a therapeutic intervention is attractive to both patients and oncologists for many reasons. The goal in treating patients is to cure their cancer while rendering a meaningful quality of life. In radiation oncology, maximizing the therapeutic index involves treating the tumor site with a high dose of radiation and minimizing the amount of normal, uninvolved tissue exposed to high radiation doses. Much work is actively proceeding in an effort to elucidate genetic predictors of radiation sensitivity. Our group has previously reported the correlation of ataxia-telangiectasia mutated (*ATM*) sequence variants and the development of adverse normal tissue response after the treatment of both breast and prostate cancer (1, 2). Single nucleotide polymorphisms (SNPs) are defined as DNA sequence variants in which the minor allele occurs in at least 1% of the population and are responsible for approximately 90% of interindividual DNA sequence variation. There is budding evidence implicating polymorphisms as risk factors for developing prostate cancer as well as the response to androgen deprivation therapy (3, 4).

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Single nucleotide polymorphisms are being increasingly studied to investigate how small genotypic variants in the population affect individual patient responses to radiotherapy (5–12).

Transforming growth factor β1 (TGF-β1), the protein encoded by *TGFB1*, is a multifunctional cytokine produced primarily by endothelial, hematopoietic, and connective tissue cells and is implicated in radiotherapy response. Virtually all human cells have receptors for TGF-β1, which regulates various cell functions, such as proliferation, differentiation, embryonic development, angiogenesis, and wound healing (13). Dysfunction of TGF-β1 has been observed in various disease states, including lung, liver, and kidney fibrosis, as well as hypertension (13). Recently, Zacchigna *et al.* (14) have demonstrated that excess TGF-β1 caused blood vessel stenosis and contributed to the development of hypertension in mice through increased peripheral vascular resistance. Previous groups have hypothesized that the TGF-β1 promoter phenotype influences TGF-β1 plasma levels (15). Grainger *et al.* studied two SNPs upstream from the main transcriptional start site: one at −509, where either a cytosine or thymine is present, and the other at position −800, where there is either an adenine or guanine. The −509 C/T genotype in which the patients were heterozygous at this allele was significantly associated with elevated plasma concentrations of TGF-β1. Others have followed, elucidating additional SNPs correlating with elevated TGF-β1 levels (8, 11).

In irradiated cells, TGF-β1 is a key cytokine associated with proliferation, differentiation, and deposition of extracellular matrix proteins (16). There is considerable evidence that TGF-β1 acts as a key mediator of fibrosis, both recruiting inflammatory cells as well as activating fibroblasts to produce extracellular matrix. Transforming growth factor β1 is central in the mitigation of postirradiation injury in various normal tissues and tumor cells (10, 17). It has been observed that a dose as low as 0.1 Gy of ionizing radiation directly induces TGF-β1 activation in less than 1 h (18, 19). In addition, ionizing radiation indirectly activates TGF-β1 by damaging endothelial cells and altering the homeostasis of reactive oxygen and nitrogen species (9). Elevated plasma TGF-β1 levels have been studied in the setting of thoracic radiation for non–small-cell lung cancer as a means of predicting patients at risk for developing radiation-induced pulmonary fibrosis (20, 21). Quarmby *et al.* (12) have reported on a series of 103 breast cancer patients who received radiotherapy and correlated TGF-β1 SNPs at the −509 and 869 positions with the development of severe radiation-induced normal tissue fibrosis. They found patients with either the −509 T/T or 869 C/C genotypes to be 7 and 15 times more likely to develop severe fibrosis, respectively. In a validation study of 167 breast cancer patients treated with radiotherapy, Giotopoulos *et al.* (22) reported that possession of the *TGFB1* −509 T/T genotype was associated with a roughly ninefold increase for the development of fibrosis compared with patients who have the *TGFB1* −509 C/C genotype. Andreassen *et al.* (6) have shown *TGFB1* polymorphisms were associated with late normal tissue skin toxicity in women treated with radiotherapy for early breast cancer. In a study of postmastectomy radiotherapy patients, the aforementioned investigators failed to replicate the initial results indicative of an association between *TGFB1* SNPs and the development of subcutaneous fibrosis (23). However, the different types of breast cancer patients and treatments should be noted, as well as the use of DNA derived from archived formalin-fixed, paraffin-embedded tissue samples for the second study compared with the use of DNA isolated from cultured fibroblasts for the initial study. Both of these differences between their studies could help to account for the contradictory results obtained in their two series.

Because of the growing evidence of the correlation between possession of certain *TGFB1* genotypes and the development of adverse normal tissue response after radiotherapy, we investigated the role of *TGFB1* SNPs in the setting of prostate cancer. We hypothesized that certain candidate *TGFB1* SNPs that result in the −509 T/T, 869 C/C (pro/pro, codon 10), and 915 G/C (arg/pro, codon 25) genotypes may be involved in the development of adverse normal tissue response after radiation. In line with previous findings in breast and lung cancer patients, these genotypes may predispose patients to adverse effects resulting from treatment of prostate cancer with radiation. The purpose of this study was to investigate whether the presence of SNPs located within *TGFB1* might be predictive for the development of adverse quality-of-life outcomes in prostate cancer patients treated with radiotherapy. We explore the potential association between possession of certain *TGFB1* genotypes and the development of three common quality-of-life measures relevant in men treated for prostate cancer: erectile dysfunction, urinary morbidity, and rectal bleeding.

METHODS AND MATERIALS

Patients

Peripheral blood lymphocytes were collected from a consecutive series of 141 patients treated at Mount Sinai Hospital for organ-confined prostate cancer between 1997 and 2005. All patients had biopsy-proven adenocarcinoma of the prostate, with central pathology review performed on all specimens. Patients were staged according to the American Joint Committee on Cancer standard (24). Patient and tumor characteristics are outlined in Table 1.

All but 1 patient was treated with low-dose-rate prostate brachytherapy using a real-time ultrasound-guided technique (25). One patient was treated with salvage external beam radiotherapy after a radical prostatectomy biochemical failure, and 1 patient was treated with a salvage partial ^{103}Pd implant 5 years after external beam radiotherapy. Treatment regimens evolved over time, thus there was overlap among different risk groups being treated by different regimens. Details of the development for these treatment schemas have been previously described (26). The implant prescription dose was 160 Gy (Task Group Report 43) for ^{125}I implants, 124 Gy (National Institute of Standards and Technology Report 99) for full ^{103}Pd implants, and 100 Gy (National Institute of Standards and Technology Report 99) for partial ^{103}Pd implants. Generally, patients at higher risk for extracapsular extension on the basis of pretreatment risk factors underwent partial (67%) dose implantation followed by external beam radiation to 45 Gy. A summary of the

Table 1. Baseline patient and clinical tumor characteristics

Age (y), median (range)	66 (46–79)
Race	
White	109 (77)
African American	20 (14)
Hispanic	8 (6)
Other	4 (3)
Erectile function	
3 (optimal)	68 (48)
2 (suboptimal but sufficient)	29 (21)
1 (suboptimal)	18 (13)
0 (none)	23 (16)
Unknown	3 (2)
IPSS score	
Good (0–7)	87 (62)
Moderate (8–19)	38 (27)
Severe (20–35)	4 (3)
Unknown	12 (8)
Urine QOL	
0–3	120 (85)
4–6	8 (6)
Unknown	13 (9)
Diabetes mellitus	9 (6)
Coronary artery disease	15 (11)
Smoking history	47 (33)
Active smoker	14 (10)
Former smoker	33 (23)
History of TURP	7 (5)
Gleason score	
≤6	115 (82)
7	18 (13)
8–10	8 (5)
Stage (AJCC 2002)	
≤T2a	114 (81.5)
T2b	19 (13)
T2c	6 (4)
Recurrent	2 (1.5)
PSA (ng/mL), median (range)	6.3 (0.07–43)
≤10	120 (85)
10–20	15 (11)
>20	6 (4)

Abbreviations: IPSS = International Prostate Symptom Score; Urine QOL = quality of life on the IPSS score; TURP = transurethral resection of prostate.

Values are number (percentage) unless otherwise noted.

Table 2. Treatment regimens and dosimetric parameters

Implant alone	109 (77)
Combined EBRT + implant	31 (22)
EBRT-alone salvage	1 (<1)
Hormone therapy	61 (43)
Implant type	
^{125}I	106 (76)
Partial ^{103}Pd	32 (23)
Full ^{103}Pd	2 (1)
Total BED (Gy_2)	197 (133–287)
EBRT dose (Gy)	45 (39.6–70.2)
Total activity (mCi)	
^{125}I	42 (23–79)
Partial ^{103}Pd	146 (60–300)
Full ^{103}Pd	221 (109–333)
D90 prostate (Gy)	
^{125}I	193 (133–239)
Partial ^{103}Pd	101 (49–144)
Full ^{103}Pd	135 (115–155)
D30 urethra (Gy)	
^{125}I	235 (164–419)
Partial ^{103}Pd	123 (78–204)
Full ^{103}Pd	166 (147–184)
V100 rectum (cm^3)	1.03 (0.01–3.04)

Abbreviations: EBRT = external beam radiotherapy; BED, biologic effective dose (grays using an $\alpha/\beta = 2$ for prostate [Gy_2]).

Values are number (percentage) or median (range).

treatment regimens and dosimetric information are presented in Table 2. All patients underwent computerized tomography–based, postimplant dose evaluation at 1 month. In an effort to compare different treatment regimens (*i.e.*, combined implant with external beam radiotherapy) and different isotopes, we calculated the biologic effective doses (BED) for each patient, as previously described (27). The median BED for the entire population was 197 Gy_2 (range, 133–287 Gy_2).

Definition of adverse response

Clinical data were available from the departmental prostate cancer database, which prospectively collected data for the 2,643 patients who underwent prostate radiotherapy at Mount Sinai between June 1990 and February 2006. All patients underwent a detailed history and physical examination before implantation, followed by a directed history and physical examination at 6-month-interval follow-up evaluations. Acute and late rectal toxicities were graded according to Radiation Therapy Oncology Group (RTOG) morbidity criteria (28). Patients who developed either RTOG Grade 1 or 2 rectal bleeding were classified as having an adverse response. Urinary tract morbidity was prospectively measured according to the American Urological Association International Prostate Symptom Score (IPSS) (29), which was administered before the implant and at each follow-up evaluation. The urinary quality-of-life score from the IPSS was used for analysis, with scores of 4 (''mostly dissatisfied''), 5 (''unhappy''), or 6 (''terrible'') for long-term urinary quality of life classified as an adverse response. Erectile function was assessed before treatment and at each follow-up evaluation using the following scoring system: 0, complete inability to have erections; 1, able to have erections but insufficient for intercourse; 2, can have erections sufficient for intercourse but considered suboptimal; and 3, normal erectile function. The derivation and relevance of this scoring system have been previously described (30, 31). Patients treated with hormonal therapy were not included in analyses of erectile function. A decline by 2 points was considered a significant decline in erection function, and these patients were classified as having an adverse response. In addition, beginning in June 2000, the validated International Index of Erectile Function (IIEF-5) was used as a complementary method to better quantify late erectile dysfunction (32). A score of 0–2 was judged as an adverse response. The last completed form was used for this study, because the relatively recent development of the IIEF-5 did not allow for a prospective evaluation in most patients.

The goals of the project were discussed with each patient, as outlined by the guidelines approved in the institutional review board protocol, and written informed consent was obtained.

TGFB1 *SNP characterization*

The lymphocyte isolation and DNA extraction procedures were performed as previously described (1). Polymerase chain reaction (PCR) primers were designed using the genomic sequence obtained from National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) and the online primer design program Primer3

(http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The optimal annealing temperature for each primer set was found using identical reaction mixes in a gradient PCR machine, which were then quantitated using a WAVE High Sensitivity DNA Fragment Analysis System (Transgenomic, Omaha, NE).

The PCR reactions were first treated with shrimp alkaline phosphatase (Promega, Madison, WI) and Exonuclease I (New England Biolabs, Ipswich, MA). The sequencing reactions (BigDye Terminator v. 3.1 Ready Reaction Cycle Sequencing kit; Applied Biosystems, Foster City, CA) were subjected to Sephadex gel filtration (Edge Biosystems, Gaithersburg, MD) and analyzed on an Applied Biosystems 3730xl DNA Analyzer.

Statistical analysis

All statistical analysis was performed by SigmaStat 3.1 software (Systat Software, Richmond, SA) and SISA online software (SISA Binomial, Southampton, UK). Univariate analysis was performed using both chi-square and Fisher exact tests, depending on the sample size. A two-sided p value of ≤ 0.05 was considered to indicate statistical significance. Odds ratios and their respective 95% confidence intervals (CIs) are also reported. Survival analysis was performed using the Kaplan-Meier actuarial method. Biochemical failure was defined according to the American Society for Therapeutic Radiology and Oncology consensus definition (33).

RESULTS

TGFB1 *polymorphisms and quality of life*

A list of the genotypes associated with the SNPs screened for in this study, along with the odds ratios for adverse response, are provided in Table 3. The −509 T/T, 869 C/C, and 915 G/C genotypes represented 8%, 16%, and 10% of the total population, respectively. For the decline in erectile function analysis, patients with pretreatment Mount Sinai Erectile Function Score of 0 or 1 were excluded. As such, 61 patients were included in this analysis. A decline in erectile function was observed in 20 of 61 (33%) of the sample. Those patients harboring the −509 T/T, 869 C/C, or 915 G/C genotypes were found to have a 56% (9 of 16) prevalence of erectile dysfunction, compared with 24% (11 of 45) for the remaining patients ($p = 0.02$). The corresponding odds ratio is 4.0 (95% CI 1.2–13.2) (Fig. 1).

A similar trend was observed for RTOG Grades 1 and 2 rectal bleeding. The rate for the entire population was 28% (40 of 141). Those patients harboring the −509 T/T genotype had a 55% (6 of 11) occurrence of rectal bleeding, compared with those who did not (26%, 34 of 130) ($p = 0.05$). The corresponding odds ratio is 3.4 (95% CI 0.97–11.82). This can be seen in Fig. 2. We observed a 3% (4 of 141) overall rate of a poor urinary quality of life, as measured by the IPSS score. There was no significant association between possessing any particular genotypes and developing adverse urinary quality of life. However, this result is likely due to the relatively small number of patients included in this study who developed urinary morbidity. Because only 4 patients in this study developed severe urinary morbidity, there was not adequate power to detect an association between any of the SNPs screened with the development of this form of radiation-induced adverse effect.

Table 3. SNP frequency and odds ratios for adverse response

SNP	Genotype	Frequency ($n = 141$) n (%)	Prospective decline in erectile function ($n = 61$) % (n)	Odds ratio (95% CI)	Late rectal bleeding ($n = 141$) % (n)	Odds ratio (95% CI)
−509 C/T	TT	11 (8)	40 (2/5)	1.41 (0.21–9.18)	55 (6/11)	3.39 (0.97–11.82)
	C/C or C/T	130 (92)	32 (18/56)	—	26 (34/130)	—
	C/T	66 (47)	34 (11/32)	1.16 (0.40–3.40)	26 (17/66)	0.78 (0.37–1.64)
	C/C or T/T	75 (53)	31 (9/29)	—	31 (23/75)	—
869 T/C	CC	22 (16)	60 (6/10)	3.96 (0.97–16.18)	36 (8/22)	1.55 (0.60–4.05)
	TT or T/C	119 (84)	27 (14/51)	—	27 (32/119)	—
	C/T	70 (50)	24 (8/33)	0.43 (0.14–1.27)	26 (18/70)	0.77 (0.37–1.61)
	C/C or T/T	71 (50	43 (12/28)	—	31 (22/71)	—
915 G/C	G/C	14 (10)	67 (4/6)	4.87 (0.81–29.33)	36 (5/14)	1.46 (0.46–4.66)
	G/G or C/C	127 (90)	29 (16/55)	—	28 (35/127)	—
	−509 T/T, 869 C/C, or 915 G/C	—	56 (9/16)	3.97 (1.20–13.18)	39 (14/36)	1.93 (0.87–4.32)
	−509 C/C, −509 C/T, 869 T/T, 869 C/T, 915 G/G, or 915 C/C	—	24 (11/45)	—	25 (26/105)	—

Abbreviations: SNP = single nucleotide polymorphism.
The SNP nomenclature is synonymous and used interchangeably throughout the text: −509TT and −509 (C>T), 869 CC, and 869 (T>C). The (−) indicates the location upstream from the promoter in the noncoding region.

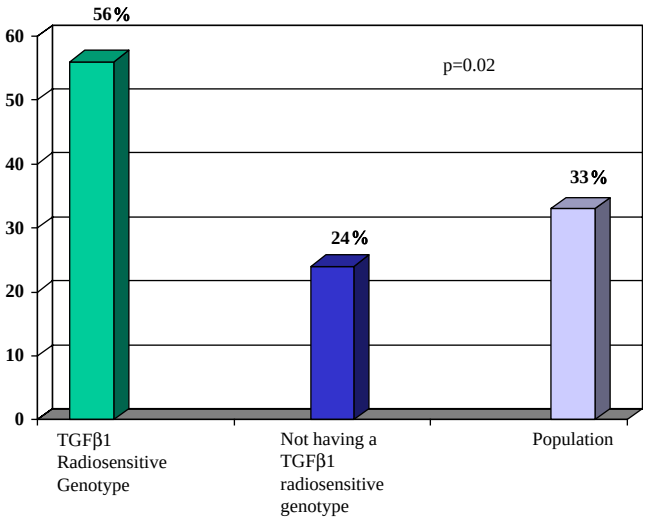

Fig. 1. Erectile dysfunction. Patients harboring single nucleotide polymorphisms in the −509TT, 869CC (T>C) (leu>pro) codon 10, or 915 (G>C) (arg>pro) codon 25 loci were found to have a 56% (9 of 16) prevalence of erectile dysfunction compared with 24% (11 of 45) for the remaining patients ($p = 0.02$). TGFβ1 = transforming growth factor β1.

We examined the effect of several other potential variables that may predict for erectile and rectal morbidity and subjected them to univariate analysis. The results can be seen in Table 4. Whereas possession of certain *TGFB1* genotypes described above significantly predicted for erectile dysfunction, other factors, such as diabetes, smoking, and having a high BED ($\geq$197 Gy_2), did not. In addition to the *TGFB1* −509 T/T genotype, smoking did correlate with developing rectal bleeding, with an odds ratio of 2.03 (95% CI 0.95–4.33) that approached statistical significance. Other factors, such as diabetes, rectal V100 $\geq$1.3 cm^3 (V100 = volume of rectum receiving 100% of the prescription dose), and BED

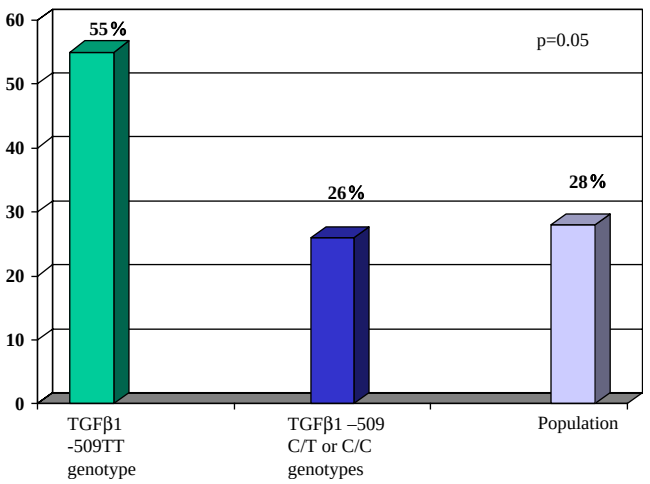

Fig. 2. Radiation Therapy Oncology Group (RTOG) late Grade 1 or 2 rectal bleeding. We observed no cases of late RTOG Grade $\geq$3 rectal bleeding. Patients harboring the −509TT polymorphism had a 55% (6 of 11) occurrence of rectal bleeding compared with those who did not 26% (34 of 130) ($p = 0.05$). TGFβ1 = transforming growth factor β1.

$\geq$197 Gy_2 did not predict for developing rectal bleeding. At last follow-up, the median prostate-specific antigen (PSA) value for the population was 0.05 ng/mL (range, 0–47 ng/mL). The actuarial 4-year freedom from PSA failure for the population was 94%.

DISCUSSION

In the United States, prostate cancer is the most common cancer in men and the third leading cause of cancer death (34). Recent patterns of care suggest that approximately 60% of men diagnosed with clinically localized prostate cancer will undergo definitive radiotherapy, either with external beam radiotherapy or brachytherapy, as their first-line treatment (35). The long-term biochemical and cause-specific survival after radiotherapy for prostate cancer patients consistently reveal tremendous efficacy in men with early-stage disease (36). Overall, radiation for localized prostate cancer is very well tolerated, and the main side effects are limited to the adjacent structures: bladder, rectum, and penis.

Maximizing the therapeutic index is of paramount importance in all fields of medicine. This is especially true in oncology when using such agents as cytotoxic chemotherapy and therapeutic radiation. There is growing interest in the radiation oncology community in the ability to predict the tumor and normal tissue response to radiation. There has been considerable success in defining dosimetric parameters that predict for erectile dysfunction, urinary morbidity, and rectal toxicity (37–46). The morbidity profiles resulting from irradiation of the rectum, urethra, bladder, and genital structures associated with both external beam radiotherapy and prostate brachytherapy have been approximated. Despite these achievements, there remains a clinically observed heterogeneity in normal tissue response that often results in frustration for the patient and clinical team. It is reasonable to hypothesize that this heterogeneity in response is largely due to genetic differences in the patient's ability to effectively respond to the consequences of radiotherapy on the cellular level. Along with other investigators, our group is attempting to identify the genetic predictors of radiation response in an emerging field of radiogenomics (47–49). In the present study we demonstrate how certain *TGFB1* genotypes may be predictive for adverse quality-of-life measures in men treated for prostate cancer.

Other investigators have identified the role of TGF-β1 in radiation-induced normal tissue injury after therapeutic irradiation (50–52). Anscher *et al*. (20, 53) have published a series of articles studying changes in plasma TGF-β1 to predict the risk of radiation-induced pneumonitis and to select patients appropriate for radiation dose escalation. More recently, their group demonstrated the amelioration of normal tissue damage caused by high-dose radiation by using an anti-TGF-β1 antibody in rats (54). Three studies have reported an association between possession of *TGFB1* SNPs and the development of late radiation effects in breast cancer patients (6, 12, 22). In addition, DeRuyck *et al*. (10) showed that −1.552delAGG, −509 C/T, and 869 T/C may be

Table 4. Variables that may predict for toxicity

Variable	OR (95% CI) for erectile dysfunction	*p*	OR (95% CI) for rectal bleeding	*p*
Having either the TGFβ1 −509 T/T, 869 C/C, or 915 G/C genotypes	3.97 (1.20–13.18)	0.02		
TGFβ1 −509 T/T genotype			3.39 (0.97–11.82)	0.05
Smoking	0.43 (0.12–1.53)	0.20	2.03 (0.95–4.33)	0.06
BED $\geq$197 Gy_2	0.52 (0.17–1.54)	0.23	0.74 (0.3–1.54)	0.4
V100 $\geq$ cm^3			1.38 (0.56–3.4)	0.4
Diabetes	0 (NA)	0.2	0.23 (0.03–2.46)	0.2
CAD	0.8 (0.14–4.5)	0.8	0.61 (0.12–3.01)	0.5
HTN	2.06 (0.65–6.48)	0.2	0.57 (0.25–1.33)	0.2

Abbreviations: OR = odds ratio; CI = confidence interval; TGFβ1 = transforming growth factor β1; BED = biologic effective dose using an $\alpha/\beta = 2$ for prostate; V100 = volume of rectum receiving 100% of the prescription dose; CAD = coronary artery disease; HTN = hypertension.

associated with higher risk of late normal tissue radiosensitivity in patients treated for gynecologic malignancies. In the present study and in those discussed above, the −509 C>T SNP has been consistently associated with the development of late radiotherapy responses. It is located in the promoter region near the nuclear hormone receptor binding site and could affect the production of the cytokine by influencing transcription (55, 56). The 869 T>C (leu>pro) and 915 G>C (arg>pro) SNPs are located in the region of the *TGFB1* gene that encodes for the portion of TGF-β1 that plays a key role in the transmembrane export of the cytokine across the endoplasmic reticulum (10).

To our knowledge, the present study is the first to explore the hypothesis that certain *TGFB1* genotypes are associated with adverse normal tissue response in prostate cancer patients treated with radiotherapy. We have shown that those patients harboring certain genotypes have an increased risk of developing adverse normal tissue quality-of-life measures in men treated with radiotherapy for prostate cancer. Specifically, men having either the −509 T/T, the 869 C/C, or the 915 G/C genotypes were significantly associated with developing a decline in erectile function. In addition, we found that patients with the −509 T/T genotype had a significantly increased risk of developing late rectal bleeding.

There are several limitations to the present study. Although the statistical analysis shows a significant association between harboring certain *TGFB1* genotypes and developing erectile dysfunction and rectal bleeding, the numbers of patients screened were limited. The risk of developing rectal bleeding is markedly elevated in patients having the −509 T/T genotype, although statistically borderline. Given more patients and longer follow-up this result may reach statistical significance. In addition, although the clinical data were prospectively collected, there is some selection bias in the retrospective nature of the analysis. Initially only patients who had particularly acute morbidities were enrolled into this study. This selection bias could explain the high (28%) occurrence of rectal bleeding for the population. This is roughly double the rate in commonly cited brachytherapy series (44, 45, 57). It is our current practice to attempt to enroll all patients to this study at the time of consultation. Therefore, future analyses may yield more accurate overall toxicity rates. Correlating treatment outcome and cancer control with *TGFB1* SNPs is not possible in this study. Because only 6% of the patients failed treatment, the study is underpowered to address this question and may not be able to detect a difference in control relative to SNP status, even if one exists.

Despite these limitations, the present study gives convincing evidence that certain *TGFB1* genotypes may be predictive of clinically meaningful adverse radiation responses in adjacent normal tissue functionality. One of our future goals is to enroll patients prospectively, collecting blood at the time of consultation to observe whether we attain similar results as those reported in this study. If congruent with our present findings, it may be possible to use *TGFB1* SNPs to predict sensitivity to therapeutic radiation, sparing the small proportion of the patient population that is radiosensitive and potentially dose-escalating the majority of patients not at high risk for development of adverse radiation response. In addition, an adequately powered study is planned, in which a sufficient number of patients who developed severe urinary morbidity after radiotherapy will be genotyped, so that it will be possible to investigate the association between *TGFB1* SNPs and this form of radiation toxicity.

It is clear that there are many additional SNPs in other genes that are associated with the development of radiation morbidity. It is, therefore, our goal to perform a genome-wide association study to identify a greater spectrum of SNPs associated with clinical radiosensitivity. This approach is now feasible with the powerful results of the HapMap project, which have identified tag SNPs that are linked to virtually all SNPs in the human genome, coupled with low-cost genotyping using high-density SNP arrays (58, 59). It is anticipated that through the performance of a genome-wide association study it will be possible to identify the SNPs that will form a basis for a predictive assay to identify patients at greatest risk for the development of adverse effects resulting from radiotherapy. Using the results of such a predictive assay, radiation oncologists will be capable of optimizing the treatment decision for each patient. In addition, identification of the genes that possess SNPs associated with clinical radiosensitivity will provide critical information essential for the

elucidation of the molecular pathways that lead to radiation injury after radiotherapy.

CONCLUSIONS

Possession of specific *TGFB1* genotypes is associated with the development of both erectile dysfunction and late rectal bleeding in patients treated with radiotherapy for prostate cancer. Future work will focus on the performance of a validation study, in which a replication set of similarly treated patients will be screened for the SNPs positively identified as associated with adverse radiotherapy effects in the present patient population. Ultimately our goal is to perform a genome-wide association study to identify the broad spectrum of SNPs and genes associated with radiation injury.

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CLINICAL INVESTIGATION **Prostate**

THERE IS NO CORRELATION BETWEEN ERECTILE DYSFUNCTION AND DOSE TO PENILE BULB AND NEUROVASCULAR BUNDLES FOLLOWING REAL-TIME LOW-DOSE-RATE PROSTATE BRACHYTHERAPY

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Purpose: We evaluated the relationship between the onset of erectile dysfunction and dose to the penile bulb and neurovascular bundles (NVBs) after real-time ultrasound-guided prostate brachytherapy.

Methods and Materials: One hundred forty-seven patients who underwent prostate brachytherapy met the following eligibility criteria: (*1*) treatment with ^{125}I brachytherapy to a prescribed dose of 160 Gy with or without hormones without supplemental external beam radiation therapy, (*2*) identification as potent before the time of implantation based on a score of 2 or higher on the physician-assigned Mount Sinai Erectile Function Score and a score of 16 or higher on the abbreviated International Index of Erectile Function patient assessment, and (*3*) minimum follow-up of 12 months. Median follow-up was 25.7 months (range, 12–47 months).

Results: The 3-year actuarial rate of impotence was 23% (34 of 147 patients). An additional 43% of potent patients (49 of 113 patients) were using a potency aid at last follow-up. The penile bulb volume receiving 100% of the prescription dose (V_{100}) ranged from 0–0.05 cc (median, 0 cc), with a dose to the hottest 5% (D_5) range of 12.5–97.9 Gy (median, 40.8 Gy). There was no correlation between penile bulb D_5 or V_{100} and postimplantation impotency on actuarial analysis. For the combined right and left NVB structures, V_{100} range was 0.3–5.1 cc (median, 1.8 cc), and V_{150} range was 0–1.5 cc (median, 0.31 cc). There was no association between NVB V_{100} or V_{150} and postimplantation impotency on actuarial analysis.

Conclusion: Penile bulb doses are low after real-time ultrasound-guided prostate brachytherapy. We found no correlation between dose to either the penile bulb or NVBs and the development of postimplantation impotency. © 2009 Elsevier Inc.

Prostate brachytherapy, Erectile dysfunction, Penile bulb, Neurovascular bundles.

INTRODUCTION

Definitive treatment options for patients with early-stage prostate cancer include surgery, external beam radiation therapy (EBRT), and brachytherapy, with similar biochemical outcomes reported in the literature (1). Evidence suggests that of these treatment modalities, brachytherapy is associated with the lowest risk of erectile dysfunction. In a meta-analysis of patients treated for localized prostate cancer, the predicted probability of maintaining erectile function at 1 year was 0.76 after brachytherapy, 0.55 after EBRT, and 0.34 after nerve-sparing radical prostatectomy (2). However, this improved rate of potency preservation may not be durable with longer follow-up (3, 4).

The cause of radiation-induced erectile dysfunction likely is multifactorial, with neurogenic, vascular, and psychogenic components. In particular, it has been proposed that dose-related damage to the penile bulb (5) and neurovascular bundles (NVBs) (6) may be causative factors. Small retrospective studies found a correlation between higher penile bulb dose and erectile dysfunction after prostate brachytherapy (5, 7). However, a large-scale cohort study of patients undergoing prostate brachytherapy did not support this finding (8). Trauma to the NVBs during radical retropubic prostatectomy was implicated as the cause of postsurgical erectile dysfunction by Walsh and Donker (9). Nevertheless, studies examining the relationship between radiation dose to the NVBs and postimplantation erectile dysfunction have consistently shown no correlation (10–12). Better understanding of the causes of treatment-related erectile dysfunction may guide improvements in brachytherapy technique, with a decrease in incidence of this side effect and improved patient quality of life.

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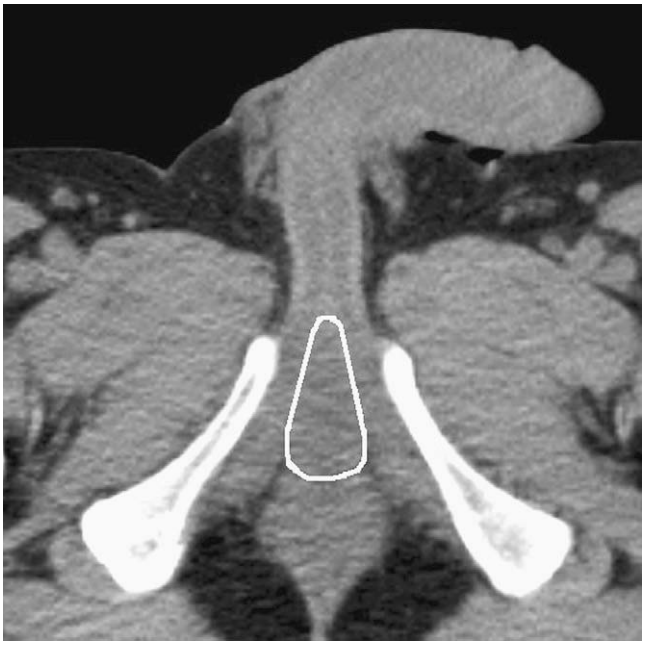

Fig. 1. The penile bulb (outlined above) was identified on axial computed tomography posterior to the corpora cavernosa, anterior to the levator ani, and between the paired lateral crura.

In this study, we retrospectively evaluated the relationship between erectile function and dose to the penile bulb and NVBs after real-time ultrasound-guided prostate brachytherapy. The NVBs are difficult to define on computed tomography (CT) imaging. Earlier studies (6, 10, 11) examining dose to these structures used an anatomic definition based on evaluation of a single cadaver by Lepor *et al*. (13), who described the location of the NVBs as 1.5–3.0 mm from the prostate capsule posterolaterally. A more recent study found that on prostate endorectal coil magnetic resonance imaging (MRI), the NVB was consistently located where the posterolateral prostate border bends away from the levator ani muscle (12), and this anatomic principle was used to reproduce NVB structures on postimplantation CT scans to enable dosimetric analysis. However, because of the location of the NVBs in an area of rapid dose fall-off, slight variations in the placement of these structures will substantially influence the calculated dose. Therefore, rather than attempt to pinpoint the exact location of the NVBs as in previous studies, we focused on dose to the fascial plane volumes that house the NVBs and postulated that higher doses to these structures might be predictive of erectile dysfunction.

METHODS AND MATERIALS

Patient population

One hundred forty-seven patients who underwent prostate brachytherapy between Jan 2003 and Feb 2006 met the following eligibility criteria: (*1*) treatment with ^{125}I brachytherapy to a prescribed dose of 160 Gy with or without hormone therapy, but without supplemental EBRT; (*2*) identification as potent before the time of implantation based on a score of 2 or higher on the physician-assigned Mount Sinai Erectile Function Score (MSEFS) and 16 or higher on the abbreviated International Index of Erectile Function (IIEF) patient assessment; and (*3*) minimum follow-up of 12 months. Median follow-up was 25.7 months (range, 12–47 months). Procedures followed were in accordance with the ethical standards of the Mount Sinai Institutional Review Board, New York, NY, and the Declaration of Helsinki of 1975, as revised in 2000.

Potency assessment

The MSEFS is a physician-assigned numerical erectile function score created at Mount Sinai Medical Center (14). Scores range from 0–3 as follows: 0 indicates no erections; 1, ability to have erections, but insufficient for vaginal penetration; 2, erectile function sufficient for vaginal penetration, but suboptimal; and 3, normal erectile function. The abbreviated IIEF is a patient-documented assessment of erectile function (15). In a prior analysis, the MSEFS was found to highly correlate with the validated patient-assessed IIEF (16).

Treatment

All patients underwent ^{125}I brachytherapy to a prescribed dose of 160 Gy for treatment of biopsy-proven prostatic adenocarcinoma. A real-time ultrasound-guided technique was used for all implantations. Details of this procedure have been described in previous publications (17, 18). No patient underwent supplemental EBRT, although hormone therapy was permitted. Indications for hormone therapy were prostate size greater than 50 cc, prostate-specific antigen level greater than 10 ng/ml, and Stage T2b or higher. Hormonal therapy was given for a 3-month duration before implantation for cytoreduction and for an additional 3 months postimplantation in patients with intermediate-risk features.

Postimplantation dosimetry

One month after implantation, patients underwent CT of the implanted area using 3.0-mm slices. This interval allowed for resolution of acute prostate swelling related to needle trauma. The CT images were imported into the VariSeed 7.1 treatment planning system (Varian Medical Systems, Inc., Palo Alto, CA). The prostate and critical structures were contoured by a radiation oncologist (R.G.S.), and the location of the brachytherapy seeds was defined by a combination of manual and automated techniques. Prostate dose was defined as the dose to the hottest 90% of the penile bulb (D_{90}) from the derived dose–volume histogram.

Contouring and dosimetry of penile bulb

On pelvic CT, the penile bulb was identified based on its anatomic relationship to nearby structures, posterior to the corpora cavernosa, anterior to the levator ani, and between the paired lateral crura (19). This anatomic relationship is shown in Fig. 1. The penile bulb was contoured on the postimplantation CT scan at 3-mm intervals by one of two physicians (R.G.S. or A.N.S.). The penile bulb volume receiving 100% of the prescription dose (V_{100}) and the penile bulb D_5 were calculated and recorded.

Contouring and dosimetry of NVBs

The NVBs are not visible on CT imaging. Rather than attempt to pinpoint their exact anatomic location, we focused on dose to the fascial plane volumes that house the NVBs. On each CT slice, the right and left NVBs and surrounding fascial planes were outlined as triangular structures bounded by the posterolateral edge of the prostate, the levator ani muscle, and the rectal wall. This method

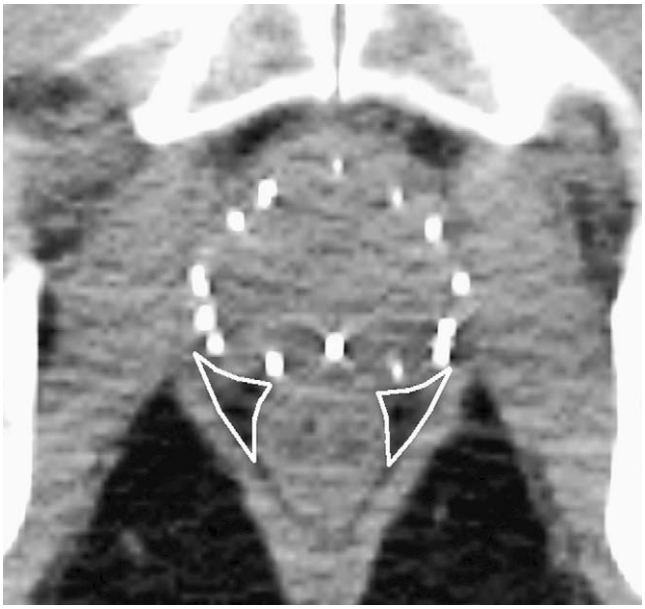

Fig. 2. Right and left neurovascular bundle structures (outlined above) were identified on axial computed tomography as triangular structures bounded by the posterolateral edge of the prostate, the levator ani muscle, and the rectal wall.

results in NVB structures as shown in Fig. 2. A single physician (A.N.S.) contoured the right and left NVB structures for all patients. For each NVB structure, NVB V_{100} and V_{150} were calculated and recorded.

Patient follow-up

Patients were seen in follow-up every 6 months after brachytherapy. At each visit, erectile function was evaluated by using the physician-assigned MSEFS, with postimplantation impotence defined as MSEFS score less than 2. Follow-up ranged from 12–47 months (median, 25.7 months).

Statistical analysis

Impotence rates were calculated using the methods of Kaplan and Meier (20). Log-rank test was used to compare rates (21, 22). Multivariate analysis was performed using Cox proportional hazard regression analysis (23).

RESULTS

Three hundred eighty-six patients underwent ^{125}I brachytherapy for treatment of localized prostate cancer between Jan 2003 and Feb 2006. Of these, 186 were identified as potent before implantation. Fifteen patients were excluded from analysis because their 1-month postimplantation CT scans did not extend far enough inferiorly to encompass the entire penile bulb. Four additional patients were excluded because postimplantation dosimetry was not available. An additional 10 patients were excluded because of insufficient follow-up. Therefore, 147 patients were the basis of our investigation.

Demographic, clinical, and treatment characteristics of the study population are listed in Table 1. Average age of the study population was 63 ± 6.9 (SD) years. The degree of pretreatment potency was full in 117 of 147 patients (80%) and partial in 30 of 147 patients (20%), defined as MSEFS scores of 3 and 2, respectively. Hormone therapy was used in conjunction with prostate brachytherapy in 37 of 147 patients (25%). For patients undergoing hormone therapy, average duration of therapy was 4.9 months (range, 3–12 months), with at least 3 months administered before implantation. Mean D_{90} for the study population was 187 ± 19 Gy. The D_{90} values ranged from 152–230 Gy (median, 187 Gy).

Posttreatment potency results for the study population are listed in Table 2. The 3-year actuarial rate of impotence was 23% (34 of 147 patients). The impotency rate was 19% (22 of 117 patients) in patients with a pretreatment MSEFS score of 3, which was considerably less than the 40% (12 of 30 patients) rate of impotence observed in patients with a pretreatment MSEFS score of 2. Of potent patients, 43% (49 of 113 patients) were using a potency aid at last follow-up, and the most commonly used agent was sildenafil. Forty-four percent of patients (42 of 95 patients) with a pretreatment MSEFS

Table 1. Demographic, clinical, and treatment characteristics (n = 147)

Age (y)	
Mean	63
Range	43–79
Race	
Caucasian	125 (85%)
Black	15 (10%)
Hispanic	5 (4%)
Asian	2 (1%)
Preimplantation erectile function	
Normal (MSEFS score of 3)	117 (80%)
Partial (MSEFS score of 2)	30 (20%)
Diabetes mellitus	
Present	12 (8%)
Absent	135 (92%)
T Stage	
T1c	118 (80%)
T2a	25 (17%)
T2b	4 (3%)
Gleason score	
6	146 (99%)
7	1 (1%)
Pretreatment prostate-specific antigen (ng/ml)	
Mean	6.0
Range	0.8–16.0
Use of hormone therapy	
Yes	37 (25%)
No	110 (75%)
Average length of hormone therapy (mo) (n = 37)	
Preimplantation	3.7
Postimplantation	1.2
Total	4.9
Prostate dosimetry	
D_{90}	
Mean (Gy)	187
Median (Gy)	187
Range (Gy)	152–230

Abbreviations: MSEFS = mount sinai erectile function score; D_{90} = dose to the hottest 90%.

Table 2. Posttreatment potency results

Potent at last follow-up (n = 147)	
Yes	113 (77%)
No	34 (23%)
Postimplantation potency aid (n = 113)	
Yes	49 (43%)
No	64 (57%)
Postimplantation potency aid type (n = 49)	
Sildenafil	40 (82%)
Tadalafil	7 (14%)
Vardenafil	2 (4%)

score of 3 were using a potency aid compared with 39% of patients (7 of 18) with a pretreatment MSEFS score of 2.

Dosimetric findings are listed in Table 3. Penile bulb volumes ranged from 1.9–18.4 cc (median, 5.7 cc). Penile bulb V_{100} values ranged from 0–0.05 cc (median, 0 cc), with a D_5 range of 12.5–97.9 Gy (median, 40.8 Gy). There was no correlation between penile bulb D_5 and postimplantation impotency on actuarial analysis (Table 4). There were no cutoff values for these parameters that predicted for a greater incidence of impotence. Volumes for the combined right and left NVB structures ranged from 4.4–27.9 cc (median, 12.7 cc). Combined NVB V_{100} values ranged from 0.3–5.1 cc (median, 1.8 cc), and V_{150} values ranged from 0–1.5 cc (median, 0.3 cc). There was no association between NVB V_{50}, V_{100}, or V_{150} and postimplantation impotency on actuarial analysis (Table 4). Additionally, there was no correlation between prostate D_{90} and postimplantation impotency on actuarial analysis (Table 4). Cox regression analysis showed no correlation between postimplantation impotency rates and age at implantation, history of diabetes mellitus, degree of pretreatment potency, hormone use, left NVB V_{100}, or right NVB V_{100}. Cox regression analysis is listed in Table 5.

Table 3. Penile bulb and neurovascular bundle dosimetric findings

Penile bulb dosimetry	
Volume (cc)	
Mean	6.27
Median	5.68
Range	1.94–18.37
V_{100} (cc)	
Mean	0.00
Median	0.00
Range	0.00–0.05
D_5 (Gy)	
Mean	43.20
Median	40.77
Range	12.50–97.97
Neurovascular bundle dosimetry	
Right	
Volume (cc)	
Mean	6.92
Median	6.54
Range	2.43–14.22
V_{100} (cc)	
Mean	1.03
Median	0.96
Range	0.07–3.35
V_{150} (cc)	
Mean	0.23
Median	0.17
Range	0.00–1.16
Left	
Volume (cc)	
Mean	6.66
Median	6.2
Range	2.00–14.88
V_{100} (cc)	
Mean	0.86
Median	0.78
Range	0.04–2.30
V_{150} (cc)	
Mean	0.17
Median	0.12
Range	0.00–0.82

Abbreviations: D_5 = dose to the hottest 5%; V_x = volume receiving x% of the prescription dose.

Table 4. Actuarial analysis

	3-y potency		
	All patients (n = 147)	No hormones (n = 110)	Hormones (n = 37)
NVB V_{50} (cc)			
≤6.0	78%	73%	68%
>6.0	67%	67%	63%
p	0.21	0.20	0.81
NVB V_{100} (cc)			
≤1.8	71%	74%	65%
>1.8	67%	66%	66%
p	0.65	0.44	0.68
NVB V_{150} (cc)			
≤0.3	75%	69%	77%
>0.3	67%	71%	60%
p	0.94	0.99	0.94
Penile bulb D_5 (Gy)			
≤40	68%	76%	54%
>40	69%	68%	86%
p	0.90	0.69	0.20
Prostate D_{90} (Gy)			
≤185	59%	59%	59%
>185	78%	78%	75%
p	0.09	0.09	0.66

Abbreviations: NVB = combined neurovascular bundles; D_x = dose to the hottest x%; V_x = volume receiving x% of the prescription dose.

To address the issue of hormone therapy as a possible confounder to our study of erectile dysfunction, we performed a separate analysis of patients treated with and without hormone therapy. Demographic, clinical, treatment, and dosimetric characteristics were similar to those presented for our entire patient population. For patients treated without hormone therapy (n = 110), average age was 62 ± 7.1 years. The degree of pretreatment potency was full in 91 of 110 patients (83%) and partial in 19 of 110 patients (17%). Mean D_{90} was 188 ± 20 Gy. The 3-year actuarial rate of impotence was 21% (23 of 110 patients). Penile bulb V_{100} values ranged from 0–0.05 cc (median, 0 cc), with a D_5 value range of 15.8–97.9 Gy (median, 41.0 Gy). Volumes for the combined

Table 5. Cox regression analysis of factors that may affect potency

Variable	*p*	Exp(B)	95% CI for Exp(B)
Age	0.33	1.03	0.97–1.09
Diabetes mellitus	0.32	1.76	0.58–5.31
Pretreatment potency	0.11	0.52	0.23–1.16
Hormone use	0.83	1.09	0.50–2.36
Right neurovascular bundle V_{100}	0.51	1.22	0.68–2.21
Left neurovascular bundle V_{100}	0.47	1.35	0.60–3.03

Abbreviations: CI = confidence interval; V_{100} = volume receiving 100% of the prescription dose.

right and left NVB structures ranged from 4.4–27.9 cc (median, 13.0 cc). Combined NVB V_{100} value range was 0.3–5.1 cc (median, 1.7 cc), and V_{150} value range was 0–1.5 cc (median, 0.3 cc). When patients treated with and without hormone therapy were analyzed separately, there was no correlation between penile bulb D_5, NVB V_{50}, NVB V_{100}, or NVB V_{150} and postimplantation impotency on actuarial analysis (Table 4).

DISCUSSION

Radiation-associated erectile dysfunction is well documented, but poorly understood. Because the prospect of maintaining potency is an important factor in a potent patient's selection of a treatment modality, it is appropriate that radiation oncologists determine whether its incidence can be reduced by optimizing the dosimetry of our interventions. Dose-related damage to the penile bulb has been implicated previously, and several investigations of erectile dysfunction after EBRT have shown a positive relationship between penile bulb dose and impotence (24–27). One study by Roach *et al*. (26) reported on 158 potent patients who were enrolled in Radiation Therapy Oncology Group 9406, a Phase I/II dose escalation study for men with localized prostate cancer (T1–T3) treated with three-dimensional conformal radiotherapy with curative intent. The risk of impotence was found to be greater in patients with a median penile bulb dose of 52.5 Gy or greater compared with patients receiving lower doses. The observation that higher doses to the penile bulb predicted for erectile dysfunction led to inclusion of the penile bulb as an avoidance structure in subsequent Radiation Therapy Oncology Group studies of EBRT for patients with prostate cancer. Conversely, data supporting a correlation between penile bulb dose and the development of erectile dysfunction after prostate brachytherapy are less convincing.

In 2001, Merrick *et al*. (5) reported a comparison of 23 men who developed erectile dysfunction after treatment of clinical stage T1/T2 prostatic adenocarcinoma with brachytherapy alone with 23 similar men who remained potent after implantation. Potency was defined simply as ''an erection sufficient for vaginal penetration'' (5). Multivariate analysis showed that dose to the penile bulb, as defined on the Day 0 postimplantation CT scan, correlated with the development of erectile dysfunction. Penile bulb D_{50} exceeding 50 Gy was present in 19 of 23 patients with erectile dysfunction and only 8 of 23 patients without erectile dysfunction. Similarly, 19 of 23 patients with erectile dysfunction had a penile bulb D_{95} exceeding 20 Gy compared with 7 of 23 patients without erectile dysfunction.

Merrick *et al*. (7) published a similar study in 2002 comparing 30 patients who became impotent after treatment with brachytherapy alone with 30 similar patients who retained potency. This analysis more specifically defined potency as a score of 11 or higher on the IIEF patient assessment. The results confirmed the positive relationship between penile bulb dose and postimplantation erectile dysfunction seen in the 2001 publication.

More recently, a large-scale cohort study examining the correlation between postimplantation impotence and penile bulb dose was published by Macdonald *et al*. (8). Three hundred forty-two patients with prostatic adenocarcinoma who were potent before therapy were treated with brachytherapy with or without hormone therapy. Postimplantation potency was evaluated by using both physician assessment and patient-documented quality-of-life questionnaires. Physician-documented rates of erectile dysfunction were 57%, 48%, and 38% at 1, 2, and 3 years after implantation, respectively. Patient-documented rates of erectile dysfunction were 70% and 66% at 1 and 2 years, respectively. Mean penile bulb D_{50} and D_{95} values were 47.8 and 23.7 Gy, respectively. In contrast to the findings of Merrick *et al*. (5, 7), there was no association between penile bulb D_{50} and D_{95} and postimplantation impotence. The number of needles used at implantation and the institutional case sequence number predicted for the development of erectile dysfunction, suggesting a traumatic cause for postimplantation impotence.

At Mount Sinai, we use a real-time ultrasound-guided implantation technique that results in narrower extraprostatic implantation margins and lower penile bulb doses than seen in previous studies. We therefore chose to look at D_5 rather than D_{50} or D_{95} values to maximize our ability to find a correlation with the development of erectile dysfunction. The mean penile bulb volume in our study was 6.27 cc, which is consistent with mean volumes reported in previous investigations by Merrick *et al*. (7) (7.6 cc) and Macdonald *et al*. (8) (6.83 cc). In our patient cohort, penile bulb D_5 value range was 12.5–97.9 Gy (median, 40.8 Gy), with a V_{100} value range of 0–0.05 cc (median, 0 cc). Given such low radiation doses to the penile bulb, it is not surprising that we failed to see a correlation between dose and postimplantation impotence. Although there was no dosimetric correlate in our patients with low penile bulb doses, it should be emphasized that this does not rule out the possibility of a dose–response relationship between the penile bulb and impotence in patients receiving higher penile bulb doses. Despite low penile bulb doses in our patients, a subset experienced erectile dysfunction, and we are attempting to uncover other possible causative factors.

Based on their examination of autonomic innervation of the corpora cavernosa in the male fetus and newborn, Walsh and Donker (9) concluded that damage to the NVBs during radical retropubic prostatectomy was responsible for the development of impotence. This discovery paved the way for the introduction of nerve-sparing surgical techniques that offered improved potency rates (28, 29). This surgical observation prompted radiation oncologists to examine the relationship between dose-related damage to the NVBs and erectile dysfunction after prostate brachytherapy. To date, no study has shown a clear correlation between NVB dose and postimplantation erectile dysfunction, although studies included only a small number of patients and efforts were impeded by the inability to visualize the NVBs on CT.

In 2000, DiBiase *et al.* (6) presented their findings for 14 patients who underwent prostate brachytherapy with or without EBRT (2 of 14 patients). The NVBs were defined on Day 0 postimplantation CT as points located 2 mm posterolateral to the prostatic capsule, based on the description by Lepor *et al.* (13). Considerable variation was observed in average NVB doses, which ranged from 130–226% of the prescription dose (which was 140 Gy). Of the 3 patients who developed early postimplantation impotence, all had received maximal NVB doses substantially higher than average.

Merrick *et al.* (10) reported 33 patients who developed erectile dysfunction after treatment with brachytherapy with or without EBRT and compared them with 21 similar patients who had retained potency. Delineation of the NVBs on Day 0 postimplantation CT was performed using a modification of the technique previously described by DiBiase *et al.* (6). By defining on each 5-mm prostate slice two additional points that lie 2 mm lateral to the original central point on an axis parallel to the posterolateral prostate border, each NVB was considered as a two-dimensional surface rather than a line. The overall mean NVB dose was 217% ± 55% of the prescribed brachytherapy dose, with no correlation identified between NVB dose and the development of postimplantation impotence.

Kiteley *et al.* (11) used the method of DiBiase *et al.* (6) to locate the NVBs of 50 men treated with ^{125}I brachytherapy alone. Mean NVB D_{50} was 158 Gy (range, 76–240 Gy). At a median follow-up of 34 months, 20 of 50 patients (40%) were impotent, and these patients were not found to have higher NVB doses compared with patients who retained potency.

Wright *et al.* (12) recognized the limitations of relying on a single cadaver study to locate the NVBs on CT. The investigators attempted to identify a more reliable method for NVB localization by analyzing nine prostate endorectal coil MRI scans. They observed that on MRI, the NVBs are consistently located where the posterolateral prostate border bends away from the levator ani muscle. This principle was used to reproduce three-dimensional NVB structures on postimplantation CT scans of 41 patients treated with implantation alone. At a median follow-up of 20 months, 11 of 41 patients (27%) were impotent. Median D_{50} values to the right and left NVBs were 124% and 106% of the prescribed dose, respectively. Their rate of erectile dysfunction was not increased in patients with higher doses to the NVBs.

Although the localization technique used by Wright *et al.* (12) may be more precise than methods used in previous studies, because the NVBs are located in an area of rapid dose fall-off, slight variations in their placement will substantially influence calculated dose. Furthermore, anatomic studies have identified additional nerve fibers in the lateral prostatic fascia that appear to have an important role in maintaining erectile function (30–32). This finding has led to the development of the Veil of Aphrodite surgical technique, which is designed to spare the lateral prostatic fascia during radical prostatectomy in the hope of improving potency rates (33, 34). The presence of these additional nerve fibers is another possible confounding variable in previous studies that used pinpoint localization of the NVBs. Therefore, rather than attempt to identify the exact location of the NVBs, we focused on dose to the fascial plane volumes that house them and postulated that higher doses to these structures might be predictive of erectile dysfunction.

Our study is the largest to investigate the relationship between dose to the NVBs and postimplantation potency. For the combined right and left NVB structures, V_{100} values ranged from 0.3–5.1 cc (median, 1.8 cc), and V_{150} values ranged from 0–1.5 cc (median, 0.31 cc). We found no association between NVB V_{100} or V_{150} and postimplantation impotence on actuarial analysis. Such a dose response may exist, but our implantations were too consistent with respect to technique and delivered dose during the selected period to detect such a relationship. Furthermore, the inability to visualize the NVBs on CT continues to be a limitation. In our study, the right and left NVBs and surrounding fascial planes were outlined as triangular structures bounded by the posterolateral edge of the prostate, the levator ani muscle, and the rectal wall. The combined volume of the right and left NVBs ranged from 4.4–27.9 cc. It seems plausible that this natural anatomic variation in size of the contoured region between individuals may have influenced our dosimetric analysis.

CONCLUSION

Penile bulb doses are consistently low after real-time ultrasound-guided prostate brachytherapy when the goal of therapy is to place every source inside the prostate gland. Nonetheless, a number of our patients developed erectile dysfunction, and we conclude that there is another mechanism responsible for brachytherapy-induced impotence. Dose-related damage to the NVBs is a logical candidate. However, despite generously contoured NVB volumes that would be expected to exaggerate findings of very high doses, our study also failed to show a correlation between NVB dose and postimplantation erectile dysfunction.

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